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CRUZ, Antonio [CA/CA]; 89 Dunloe Road, Toronto, On-  
tario M5P 2T7 (CA).

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(74) Agents: KURDYDYK, Linda et al.; Suite 4700, Box 48,  
66 Wellington Street West, Toronto Dominion Bank Tower,  
Toronto, Ontario M5K 1E6 (CA).

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(71) Applicant (for all designated States except US):  
WARATAH PHARMACEUTICALS, INC. [CA/CA];  
415 Yonge Street, Suite 1103, Toronto, Ontario M5B 2E7  
(CA).

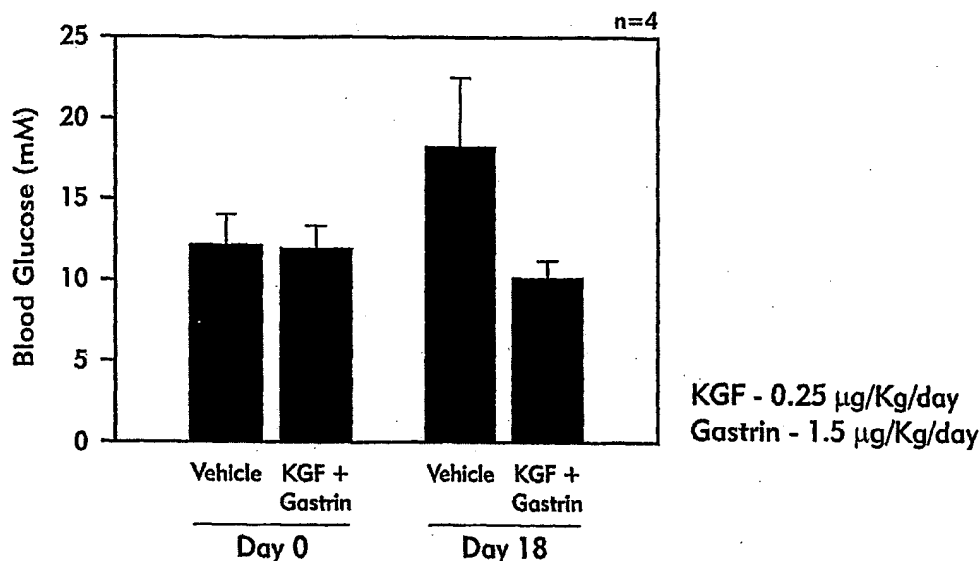
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(72) Inventors; and

(75) Inventors/Applicants (for US only): BRAND, Stephen J,  
[AU/US]; 161 Bedford Road, Lincoln, MA 01773 (US).

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(54) Title: COMBINED USE OF KERATINOCYTE GROWTH FACTOR AGONISTS AND GASTRIN COMPOUNDS



(57) Abstract: The invention relates generally to compositions, conjugates, and methods comprising a KGF agonist and a gastrin compound. The compositions can be used in the treatment and/or prevention of conditions for which either a KGF agonist or a gastrin compound have been demonstrated to have a therapeutic effect, including but not limited to diabetes, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, and obesity.



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**Title: Combined Use of Keratinocyte Growth Factor Agonists and Gastrin Compounds**

**FIELD OF THE INVENTION**

The invention relates generally to compositions, conjugates, and methods comprising a keratinocyte growth factor agonist and a gastrin compound, and uses thereof.

**5 BACKGROUND OF THE INVENTION**

The fibroblast growth factor (FGF) family consists of at least 22 structurally related polypeptides (named FGF-1 to FGF-22). The members of the FGF family typically range in molecular mass from 17 to 34 kDa and share 13-71% amino acid identity. FGFs are highly conserved in both gene structure and amino-acid sequence among vertebrate species. The various FGF molecules exhibit a broad range of biological  
10 activities in normal and malignant conditions. These activities include angiogenesis, mitogenesis, cellular differentiation, and wound repair (Baird, A. et al., *Cancer Cells* 3:239-243 (1991); Burgess, W. H. et al., *Annu. Rev. Biochem.* 58:575-606 (1989)). When inappropriately expressed, some FGFs can contribute to the pathogenesis of cancer.

Keratinocyte growth factor (KGF, KGF-1, or FGF-7) is a member of the FGF family but unlike  
15 other members of the FGF family it has little activity on mesenchyme-derived cells but stimulates epithelial cells. KGF has been reported to produce changes in hair follicle morphogenesis, hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine (Panos et al *J. Clin. Invest* 92:969-977 (1993); Ulich et al., *Am J. Path.* 144:862-868 1994; Yi et al, *Am J Path* 145:80-85, 1994; and Ulich et al., *J. Clin Invest.* 93:1298-1306, 1994). Keratinocyte growth factor 2  
20 (KGF-2, FGF-10) is a 208 amino acid glycoprotein that has a high protein sequence similarity to FGF-7, and they are both mitogenic for keratinocytes (Emoto et al, *J. Biol. Chem.* 272 23191-23194). (See Powers C.J. et al, *Endocrine-Related Cancer* 7:165-197, 2000 for a review of fibroblast growth factors).

KGF administered systemically has been reported to give partial reduction of elevated blood glucose in streptozotocin induced diabetic rats, however, this effect is confined to KGF administered within  
25 1-2 days of the streptozotocin (STZ) (U.S. Patent Number 5,858,977, issued Jan. 12, 1999). The amelioration of diabetes may result from KGF protecting  $\beta$  cells from the toxicity of streptozotocin. Thus, this method may have limited utility, as the loss of  $\beta$  cells for most diabetics has occurred long before initiation of treatment. Furthermore, KGF was effective in reducing blood glucose only in moderately diabetic rats. KGF administration resulted in long term reduction in blood glucose only if non-fasting blood  
30 glucose was below 15mM. More severely STZ diabetic rats, with blood glucose greater than 15mM, responded with only a transient reduction in blood glucose, and only a modest increase in pancreatic insulin content. Since no increase in plasma insulin was demonstrated, the observed increased pancreatic insulin may be a consequence of the lower blood glucose reducing insulin release from the pancreas. No quantitative histological data was reported showing increased  $\beta$  cell mass in response to administration of KGF. Thus, the  
35 improvement in glucose tolerance after KGF treatment in STZ diabetic rats was not conclusively demonstrated to result from stimulation of  $\beta$  cell regeneration. Therefore, KGF was of low potency for producing an anti-diabetic response. High doses of KGF were required to improve glucose tolerance, i.e. doses below 1mg/kg had no effect on blood glucose levels. The doses of KGF required to reduce blood glucose in diabetic rats further caused significant proliferation of the liver and other tissues.

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The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

#### SUMMARY OF THE INVENTION

5 The present invention is directed to a combination of a keratinocyte growth factor (KGF) agonist and a gastrin compound that provides beneficial effects in the treatment of conditions for which either a KGF agonist or a gastrin compound have been demonstrated to have a therapeutic effect, including but not limited to diabetes, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, and obesity. Combinations of a KGF agonist and a gastrin compound may be selected to provide unexpectedly additive effects or greater than additive effects i.e. synergistic effects.

10 A composition, conjugate, or combination therapy comprising a KGF agonist and a gastrin compound employing different mechanisms to achieve maximum therapeutic efficacy, may improve tolerance to the therapy with a reduced risk of side effects that may result from higher doses or longer term monotherapies (i.e. therapies with each compound alone). A composition or combination treatment of the invention can permit the use of lower doses of each compound with reduced adverse toxic effects of each compound. A suboptimal dosage may provide an increased margin of safety, and may also reduce the cost of a drug necessary to achieve prophylaxis and therapy. In addition, a treatment utilizing a single combination dosage unit will provide increased convenience and may result in enhanced compliance.

15 The invention contemplates a composition, preferably a pharmaceutical composition, comprising a KGF agonist and a gastrin compound. A pharmaceutical composition may optionally comprise a pharmaceutically acceptable carrier, excipient, or vehicle.

In an aspect, the composition provides beneficial effects relative to each compound alone. In another aspect, the invention contemplates a pharmaceutical composition comprising a KGF agonist and a gastrin compound which provides beneficial effects, preferably sustained beneficial effects, following treatment.

25 The beneficial effects provided by a composition of the invention can include increased absorption, distribution, metabolism and/or elimination of KGF agonist and/or gastrin compound. A composition can have increased bioavailability (absorbed more rapidly and to a higher degree) or provide enhanced therapeutic effects.

30 The invention also provides a pharmaceutical composition for the treatment of a disease or condition comprising a therapeutically effective amount of a KGF agonist and a gastrin compound in a pharmaceutically acceptable carrier, excipient, or vehicle.

In another aspect, the invention features a composition comprising a gastrin compound and a KGF agonist. The composition is in a dosage effective for inducing proliferation of islet precursor cells into an increased amount of mature insulin secreting cells. Further, the composition is in a dosage effective for inducing differentiation of an islet precursor cell into a mature insulin secreting cell. The composition can be in a pharmaceutically acceptable carrier.

35 The invention provides a conjugate comprising a KGF agonist linked to a gastrin compound. A conjugate can provide the beneficial effects described herein.

40 The invention also provides methods for preparing compositions and conjugates of the invention that result in compositions and conjugates with beneficial effects.

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In an aspect the invention provides a method of preparing a stable pharmaceutical composition of a KGF agonist adapted to provide beneficial effects following treatment, comprising preparing a composition comprising the KGF agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the KGF agonist and/or gastrin compound.

5       The invention also contemplates the use of a composition or conjugate of the invention or combination treatment of the invention for preventing, and/or ameliorating disease severity, disease symptoms, and/or periodicity of recurrence of a disease or condition described herein. The invention also relates to the prevention and treatment, in a subject, of conditions or diseases using the compositions, combination treatments, and conjugates of the invention.

10       The invention provides a method for treating and/or preventing a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one KGF agonist and at least one gastrin compound to provide beneficial effects. In an aspect the invention provides a treatment method which provides sustained beneficial effects following treatment.

15       The invention provides a method of treating a condition or disease comprising administering a KGF agonist and a gastrin compound, a composition, or conjugate of the invention, with a plurality of cells, to a subject in need thereof to thereby produce beneficial effects. In an embodiment, the compounds/composition/conjugate are administered systemically.

20       The invention provides a method for expanding and differentiating stem cells or progenitor cells into insulin secreting cells comprising contacting the stem cells or progenitor cells with a KGF agonist and a gastrin compound, or a composition or conjugate of the invention in sufficient amounts to expand and differentiate stem cells or progenitor cells. The amount of expansion and differentiation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate. In an embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in culture. In another embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in a subject. The compounds, composition or conjugate may be administered to a subject before, during, or after implantation of stem cells in the subject to expand and differentiate the stem cells in the subject.

25       The invention also relates to a method for enhancing proliferation of insulin secreting cells in culture comprising contacting the cells with a KGF agonist and a gastrin compound, or a composition or conjugate of the invention, in sufficient amounts to enhance proliferation of the cells. The amount of proliferation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate.

30       The invention also relates to a method for sustaining islet cells or precursor cells in culture comprising culturing the cells in the presence of a KGF agonist and a gastrin compound or a composition or conjugate of the invention in an amount sufficient to sustain the cells in culture. The cells may be sustained in culture for a significantly longer period of time compared with cells cultured in the absence of the compounds, composition or conjugate. Culturing cells in the presence of a KGF agonist and a gastrin compound or a composition or conjugate of the invention will be particularly useful in preparing and maintaining cells intended for transplantation.

The invention further relates to a method for treating a subject with a condition or disease described herein comprising contacting *ex vivo* a plurality of cells with a KGF agonist and a gastrin compound, or a composition or conjugate of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

5       The invention still further relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with a KGF agonist and a gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

10       The invention provides methods for treating diabetes mellitus in a patient in need thereof by administering a composition comprising a gastrin compound and a KGF agonist in an amount sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin synthesis in existing islet cells. The composition can be administered systemically or expressed *in situ* by host cells containing a nucleic acid construct in an expression vector wherein the nucleic acid construct comprises a coding sequence for a gastrin compound and/or a coding sequence for a KGF  
15       agonist, together with transcriptional and translational regulatory regions functional in pancreatic islet precursor cells.

Also provided are methods and compositions for treating diabetes in a patient in need thereof by implanting into a diabetic patient pancreatic islet cells that have been exposed in culture to a sufficient amount of a gastrin compound and a KGF agonist to increase the number of pancreatic beta cells in the  
20       islets; optionally the population of pancreatic beta cells can be grown in culture for a time sufficient to expand the population of  $\beta$ -cells prior to transplantation.

The invention also contemplates the use of a composition comprising a combination of at least one KGF agonist and at least one gastrin compound for the preparation of a medicament for preventing and/or treating a condition or disease. In an embodiment, the invention relates to the use of synergistically effective  
25       amounts of at least one KGF agonist, and at least one gastrin compound for the preparation of a medicament for preventing and/or treating a condition or disease. The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for the prevention and/or treatment of conditions and diseases. The medicaments provide beneficial effects, preferably sustained beneficial effects following treatment.

30       The invention in an embodiment provides a method for preventing and/or treating diabetes, the method comprising administering to a mammal in need thereof a composition comprising a combination of a KGFR ligand, such as KGF or FGF-7 or KGF-2 or FGF-10 and a gastrin compound (e.g. gastrin /CCK receptor ligand), in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal, thereby preventing and/or treating the diabetes. The composition is administered systemically. The  
35       mammal is a diabetic mammal, for example, the mammal has been diabetic for an extent of 1% of the lifespan of the mammal. The amount of KGF agonist in the composition can be substantially lower than the minimum effective dose of KGF agonist required to reduce blood glucose in the diabetic mammal in the absence of a gastrin compound. The KGF agonist and the gastrin compound are provided in an amount

sufficient to induce differentiation of the pancreatic islet precursor cells into glucose responsive insulin secreting islet cells.

Another embodiment of the invention provides a method for preventing and/or treating diabetes, the method comprising administering to a mammal in need thereof a composition comprising a combination of a  
5 KGF agonist and a gastrin compound (e.g. gastrin /CCK receptor ligand), in an amount sufficient to increase proliferation of islet precursor cells in pancreatic tissue, thereby preventing and/or treating the diabetes.

The methods of the invention can further comprise administering at least one agent for suppression of immune response, for example, the agent is selected from rapamycin, cyclosporin, ISAtx247 and FK506.

Another embodiment of the invention provides a method for preventing and/or treating diabetes, the  
10 method comprising: contacting *ex vivo* a plurality of cells with a composition comprising a KGF agonist and a gastrin compound (e.g. gastrin /CCK receptor ligand) in an amount sufficient to increase proliferation of islet precursor cells and the amount of insulin secreting islet cells; and administering the contacted plurality of cells to a mammal in need thereof, thereby preventing and/or treating the diabetes. The cells can be autologous. The composition is provided in an amount sufficient to effect differentiation of stem cells, for  
15 example, to effect differentiation of pancreatic islet precursor cells in pancreatic tissue into mature insulin secreting islet cells. The composition is provided in an amount sufficient to increase proliferation of pancreatic islet stem cells, for example, of pancreatic islet precursor cells. Stem cells can be obtained either from a pancreatic tissue or from a non-pancreatic tissue, such as liver or bone marrow.

In another aspect, an embodiment of the invention provides a method for expanding and  
20 differentiating stem cells in a diabetic recipient of the cells into insulin secreting cells, the method comprising implanting the cells in the recipient, and administering a composition containing an effective dose of each of a gastrin compound and a KGF agonist. For example, the implanted cells are obtained from a human, for example, are obtained from human pancreatic islets, human liver, human bone marrow, human umbilical cord, or human embryos. Implanting the cells into the recipient may be by a route such as injecting  
25 directly into an organ, for example, into the pancreas, the kidney, or the liver. Alternatively, implanting the cells may be administering by intravenous injection, for example, into the portal vein or into the hepatic vein. In certain embodiments, prior to implanting the cells are treated *ex vivo* with a composition comprising a gastrin compound and a KGF agonist.

In another aspect, the invention provides a method for preventing and/or treating diabetes, the  
30 method comprising administering to a mammal in need thereof a composition comprising a combination of a KGF agonist and a gastrin compound (e.g. gastrin /CCK receptor ligand), in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal; and determining the amount of islet neogenesis, thereby preventing and/or treating the diabetes. Determining the amount of islet neogenesis is measured by a parameter selected from the group of: blood glucose, serum glucose, blood glycosylated  
35 hemoglobin, pancreatic  $\beta$  cell mass, serum insulin, and pancreatic insulin content. Administering the composition reduces blood glucose compared to blood glucose assayed prior to administering the composition. Glycosylated hemoglobin concentration is reduced compared to glycosylated hemoglobin concentration in the mammal assayed prior to administering the composition. Serum insulin concentration is increased compared to serum insulin concentration in the mammal assayed prior to administering the

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composition. Pancreatic insulin concentration is increased compared to pancreatic insulin concentration in the mammal assayed prior to administering the composition.

In another aspect, the invention provides a method for inducing pancreatic islet neogenesis in a mammal, the method comprising administering to the mammal a composition comprising a combination of a KGF agonist and a gastrin compound, in an amount sufficient to increase proliferation of islet precursor cells in pancreatic tissue, thereby inducing pancreatic islet neogenesis. The plurality of cells can be multicellular. The plurality of cells are delivered systemically to the mammal.

In another aspect, the invention provides a method for inducing pancreatic islet neogenesis in a mammal, the method comprising administering a composition comprising a combination of a KGF agonist and a gastrin compound, in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal.

In another aspect, the invention provides a method for inducing islet neogenesis therapy in a cell of an animal, comprising contacting the cell with a nucleic acid sequence encoding a gastrin compound operably linked to a regulatory element, for example, an insulin promoter receptor ligand and a nucleic acid sequence encoding a KGF agonist operably linked to a regulatory element; for example, a metallothionein promoter. For example, the cell is a germ cell, or the cell is an autologous cell cultured *ex vivo*.

In another aspect, the invention provides a nucleic acid construct comprising a nucleic acid sequence encoding a mammalian KGF agonist operably linked to a heterologous promoter and a nucleic acid sequence encoding a mammalian gastrin compound operably linked to a heterologous promoter.

In another aspect, the invention provides a transgenic animal whose germ cells comprise a nucleic acid sequence encoding a mammalian KGF agonist operably linked to a heterologous promoter and a nucleic acid sequence encoding a mammalian gastrin compound operably linked to a heterologous promoter.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates or compositions, the invention also provides a kit comprising a KGF agonist and a gastrin compound, a pharmaceutical composition, or conjugate of the invention in kit form.

Therefore, in an aspect, the invention provides a kit for preventing and/or treating diabetes, containing a composition comprising a gastrin compound and a KGF agonist, a container, and instructions for use. The composition of the kit can further comprise a pharmaceutically acceptable carrier.

These and other aspects, features, and advantages of the present invention should be apparent to those skilled in the art from the following drawing and detailed description.

#### **DESCRIPTION OF THE DRAWINGS**

The invention will be better understood with reference to the drawing in which:

Figure 1 is a graph showing fasting blood glucose levels in vehicle-treated mice and in mice treated with a combination of a KGF and a gastrin.

#### **DETAILED DESCRIPTION OF EMBODIMENTS**

##### **Glossary**

Numerical ranges recited herein by endpoints include all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about." The term "about" means plus or



minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made. Further, it is to be understood that "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds.

- 5 Compounds described herein can contain one or more asymmetric centers and may give rise to enantiomers, diastereomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. Therefore, the invention includes all such possible diastereomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the
- 10 the compounds described herein contain centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. All tautomeric forms are intended to be included within the scope of the invention.

- The terms "subject", "individual", "recipient" or "patient" refer to an animal including a warm-blooded animal such as a mammal, which is afflicted with or suspected of having or being pre-disposed to a
- 15 disease or a condition described herein. Mammal includes without limitation any members of the Mammalia. In general, the terms refer to a human. The terms also include domestic animals bred for food or as pets, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals, rats, goats, apes (e.g. gorilla or chimpanzee), and rodents such as rats and mice. The methods herein for use on subjects/individuals/patients contemplate prophylactic as well as curative use. Typical subjects for treatment include persons susceptible
- 20 to, suffering from or that have suffered a condition or disease described herein. In embodiments of the invention a subject may be non-diabetic, pre-diabetic, or diabetic.

- The term "pharmaceutically acceptable carrier, excipient, or vehicle" refers to a medium which does not interfere with the effectiveness or activity of an active ingredient and which is not toxic to the hosts to which it is administered. A carrier, excipient, vehicle, or diluent includes binders, adhesives, lubricants,
- 25 disintegrates, bulking agents, wetting or emulsifying agents, pH buffering agents, and miscellaneous materials such as absorbants that may be needed in order to prepare a particular composition. Examples of carriers etc include but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The use of such media and agents for an active substance is well known in the art.

- "Pharmaceutically acceptable salt(s)," includes salts of acidic or basic groups which may be present
- 30 in the compounds suitable for use in the present invention. Examples of pharmaceutically acceptable salts include sodium, calcium, ammonium, ferric hydroxides, isopropylamine, triethylamine, 2-chylamine, 2-ethylamino, ethanol, histidine, procaine, and potassium salts of carboxylic acid groups and hydrochloride salts of amino groups. Other pharmaceutically acceptable salts of amino groups are hydrobromide, sulfate, hydrogen sulfate, phosphate, acetate, oxalic, hydrogen phosphate, dihydrogen phosphate, acetate, succinate,
- 35 citrate, tartrate, lactate, mandelate, methanesulfonate (mesylate) and p-toluenesulfonate (tosylate) salts.

The term "preventing and/or treating" refers to the administration to a subject of a composition or conjugate of the invention either before or after onset of a condition or disease. A treatment may be either performed in an acute or chronic way.

- A "beneficial effect" refers to an effect of a combination of a KGF agonist and a gastrin compound,
- 40 or composition or conjugate thereof, in particular an effect that is greater than the effect of either of the

compounds alone. The beneficial effect includes favorable pharmacological and/or therapeutic effects, and improved pharmacokinetic properties and biological activity. A beneficial effect may be an additive effect or synergistic effect.

In embodiments of the invention, beneficial effects include but are not limited to the following:  
5 reduced or absent islet inflammation, decreased or prevention of disease progression, increased survival, or treat or reverse a disease or condition.

In an embodiment, the beneficial effects can be evidenced in diabetes by one or more of the following: (a) a reduction in fasting blood glucose levels, in particular when blood glucose levels are greater than 7-10 mM; (b) reduction in glycosylated haemoglobin; (c) increase in serum insulin concentration; (d) an  
10 increase in pancreatic insulin production or content; and/or (e) prevention of disease progression. In a particular embodiment, the beneficial effects comprise (a), (b) and (c), or (a), (c), and (d).

In a preferred embodiment, the beneficial effect is a "sustained beneficial effect" where the beneficial effect is sustained for a prolonged period of time after termination of treatment. A beneficial effect may be sustained for at least about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12  
15 months, and 2 weeks to 18 months following treatment. The period of time a beneficial effect is sustained may correlate with the duration and timing of the treatment. A subject may be treated continuously for about 2 to 8 weeks, 2 to 12 weeks, 2 to 16 weeks, 2 weeks to 6 months, 2 weeks to 12 months, or periodically. A sustained beneficial effect may manifest as one or more of increased C-peptide production, increased pancreatic insulin production or concentration, and about normal or low blood glucose levels for a prolonged  
20 period following treatment.

The beneficial effect may be a statistically significant effect in terms of statistical analysis of an effect of the two compounds versus the effects of each of the compounds. "Statistically significant" or "significantly different" effects or levels with two compounds compared with each compound alone may represent levels that are higher or lower than a standard. In embodiments of the invention, the difference may  
25 be 1.5, 2, 3, 4, 5, or 6 times higher or lower compared with the effect obtained with each compound alone.

An "additive effect" of a KGF agonist and a gastrin compound refers to an effect that is equal to the sum of the effects of the two individual compounds

A "synergistic effect" of a KGF agonist and a gastrin compound refers to an effect that is greater than the additive effect which results from the sum of the effects of the two individual compounds.

30 A "combination treatment" or "administering in combination" means that the active ingredients are administered concurrently to a patient being treated. When administered in combination each component may be administered at the same time, or sequentially in any order at different points in time. Therefore, each component may be administered separately, but sufficiently close in time to provide the desired effect, in particular a beneficial, additive, or synergistic effect. The first compound may be administered in a  
35 regimen which additionally comprises treatment with the second compound.

"Therapeutically effective amount" relates to the amount or dose of active compounds (e.g. KGF agonist and gastrin compound) or compositions or conjugates of the invention that will lead to one or more desired beneficial effects, in particular, one or more sustained beneficial effects. A therapeutically effective amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the  
40 individual, and the ability of the substance to elicit a desired response in the individual. Dosage regima may

be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

5 "Suboptimal dose" or suboptimal dosage" refers to a dose or dosage of an active compound which is less than the optimal dose or dosage for that compound when used in monotherapy.

A "native-sequence polypeptide" or "a native polypeptide" comprises a polypeptide having the same amino acid sequence of a polypeptide derived from nature. Such native-sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term specifically encompasses naturally occurring truncated or secreted forms of a polypeptide, polypeptide variants including  
10 naturally occurring variant forms (e.g. alternatively spliced forms or splice variants), and naturally occurring allelic variants.

The term "polypeptide variant" means a polypeptide having at least about 70-80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95% amino acid sequence identity with a native-sequence polypeptide, in particular having at least 70-80%, 85%, 90%, 95%,  
15 98%, or 99% amino acid sequence identity to the sequences identified in any of SEQ ID NOs. 1 through 11. Such variants include, for example, polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length or mature sequences of SEQ ID NOs: 1 through 11 including variants from other species, but excludes a native-sequence polypeptide.

Percent identity of two amino acid sequences, or of two nucleic acid sequences identified herein is  
20 defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues in a polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid or nucleic acid sequence identity can be achieved in various conventional ways, for instance,  
25 using publicly available computer software including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST programs are publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990). Skilled artisans can determine appropriate parameters for measuring alignment, including any  
30 algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Methods to determine identity and similarity are codified in publicly available computer programs.

An "analog" refers to a polypeptide wherein one or more amino acid residues of a parent polypeptide have been substituted by another amino acid residue, one or more amino acid residues of a parent polypeptide have been inverted, one or more amino acid residues of the parent polypeptide have been  
35 deleted, and/or one or more amino acid residues have been added to the parent peptide. Such an addition, substitution, deletion, and/or inversion may be at either of the N-terminal or C-terminal end or within the parent polypeptide, or a combination thereof.

Mutations may be introduced into a polypeptide by standard methods, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions can be made at one or more  
40 predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which an

- 10 -

amino acid residue is replaced with an amino acid residue with a similar side chain. Amino acids with similar side chains are known in the art and include amino acids with basic side chains (e.g. Lys, Arg, His), acidic side chains (e.g. Asp, Glu), uncharged polar side chains (e.g. Gly, Asp, Glu, Ser, Thr, Tyr and Cys), nonpolar side chains (e.g. Ala, Val, Leu, Iso, Pro, Trp), beta-branched side chains (e.g. Thr, Val, Iso), and aromatic side chains (e.g. Tyr, Phe, Trp, His). Mutations can also be introduced randomly along part or all of the native sequence, for example, by saturation mutagenesis. Following mutagenesis the variant polypeptide can be recombinantly expressed.

A "derivative" refers to a polypeptide in which one or more of the amino acid residues of a parent polypeptide have been chemically modified. A chemical modification includes adding chemical moieties, creating new bonds, and removing chemical moieties. A polypeptide may be chemically modified, for example, by alkylation, acylation, glycosylation, pegylation, ester formation, deamidation, or amide formation.

A "chimeric polypeptide" comprises all or part (preferably biologically active) of a selected polypeptide operably linked to a heterologous polypeptide (i.e., a polypeptide other than the selected polypeptide). Within the fusion protein, the term "operably linked" is intended to indicate that a selected polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of a selected polypeptide. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

In the present context, a "keratinocyte growth factor agonist" or "KGF agonist" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially, directly or indirectly, potentiate, induce, mimic, or otherwise enhance the activity of keratinocyte growth factors or their receptors. In an aspect a KGF agonist fully or partially activates a human keratinocyte growth factor receptor. Without being limited by any specific mechanism, full or partial activation of a human keratinocyte growth factor receptor may thereby modulate an FGF signaling pathway and thus contribute to a condition or disease. A keratinocyte growth factor receptor includes receptors that associate with a keratinocyte growth factor, including but not limited to FGFR-2, IIIb and/or FGFR-1, IIIb.

In a preferred embodiment, a "keratinocyte growth factor agonist" or "KGF agonist" is any peptide or non-peptide small molecule or compound that associates, binds to, interacts with, or stimulates a KGF receptor ("KGF receptor ligand"), preferably with a selected affinity constant (KD) or a potency (EC<sub>50</sub>) as measured by methods known in the art.

In an aspect, a KGF agonist exhibits insulinotropic activity, where insulinotropic activity may be measured *in vivo* or *in vitro* assays known to those of ordinary skill in the art. In another aspect, a KGF agonist stimulates proliferation of keratinocytes. In an embodiment, a KGF agonist is selected that stimulates proliferative metaplasia of the pancreatic ducts, including re-activation of  $\beta$  islet cell neogenesis.

A KGF agonist includes native-sequence or synthetic polypeptides, fragments, analogs (e.g. muteins), derivatives, isoforms, chimeric polypeptides, polypeptides with sequence identity, peptidomimetics, and pharmaceutically acceptable salts thereof, and active metabolites and prodrugs.

In particular, a KGF agonist includes active analogs, fragments and other modifications, which for example share amino acid sequence identity with a native-sequence KGF agonist (e.g. KGF or KGF-2), for example, share 60%, 70%, 80%, 90%, 95%, 98%, or 99% sequence identity.

In aspects of the invention a KGF agonist is a KGF receptor ligand, in particular a keratinocyte growth factor or KGF. Keratinocyte growth factors are tyrosine kinase receptor growth factors with biological actions similar to EGF receptor ligands. KGF stimulates via a tyrosine kinase receptor different from EGF receptor, causing increased proliferation of epithelial precursors in many epithelial cell types (Krakowski, M.L. et al., Am. J. Pathol. 154:683-691, 1999). It has a unique target cell specificity. In general, KGF stimulates the proliferation and differentiation of a variety of cell types derived from the primary or secondary mesoderm as well as from neuroectoderm. KGF stimulates epithelial cell proliferation but unlike other FGF's it does not stimulate endothelial cells or fibroblast proliferation (Finch P.W. et al, Science 245: 752-755, 1989).

A KGF includes a native-sequence full length polypeptide, excluding the signal sequence, designated KGF<sub>163</sub> (KGF or FGF-7) comprising 163 amino acids, and possessing a potential N-glycosylation site at amino acid 14 of the consensus sequence for glycosylation that extends from amino acids 14 to 16 at the N-terminus (Finch P.W. et al, Science 245: 752-755, 1989). *fgf-7* codes for a 194 amino acid protein containing a signal sequence (Aaronson SA, Bottaro DP, Miki T, Ron D, Finch PW, Fleming TP, Ahn J, Taylor WG and Rubin JS 1991, Annals of the New York Academy of Sciences 638: 62-77).

An amino acid sequence for a KGF is shown in SEQ ID NOs: 1, 2, and 10, (SEQ ID NO: 10 is 163 amino acids and an initiating methionine), and includes interchangeably unless otherwise indicated, native KGF and KGF analogs (e.g. muteins) characterized by a peptide sequence substantially the same as the peptide sequence of native KGF and by retaining some or all of the biological activity of native KGF, particularly non-fibroblast epithelial cell proliferation (e.g. exhibiting at least about 500-fold greater stimulation of BALB/MK keratinocyte cells than that on NIH/3T3 fibroblast cells, and at least about 50-fold greater stimulation of BALB/MK keratinocyte cells than for BS/589 epithelial cells or for CC12098 epithelial cells, as determined by <sup>3</sup>H-thymidine incorporation).

A KGF also includes the native sequence of KGF-2 (FGF-10) which is a 208 amino acid glycoprotein with a signal sequence ((Emoto et al, 1997, supra; Yamasaki et al., J.Biol. Chem. 271:15918-15921, 1996; review article Powers et al., Endocrine-Related Cancer 7:165-197). A sequence for a native KGF-2 is shown in SEQ ID NOs: 3, 4, and 11 (SEQ ID NO: 11 is 169 amino acids and an initiating methionine).

A KGF agonist for use in the present invention includes a KGF of SEQ ID NO: 1, 2, 3, or 4 without the signal sequence (e.g. about the first 30-40 amino acid residues; see SEQ ID NOs: 10 or 11).

In a particular aspect of the invention a KGF agonist for use in the present invention is repifermin (Human Genome Sciences, Rockville, MD) or palifermin (Amgen, Thousand Oaks, Cal.).

Examples of analogs and derivatives of a KGF contemplated in the present invention are set out in Table 1 and the references cited therein which are incorporated herein by reference.

Functional assays for use in selecting a KGF agonist for use in the present invention are known in the art and include the mitogenic assays described for example in Hsu et al, Protein Expression and Purification 12:189, 1998, Rubin et al, 1989, and Igarishi et al, J. Biol. Chem. 273:13230, 1998.

A KGF agonist can be prepared by conventional methods known in the art. In particular, an amino acid portion of a KGF agonist can be prepared by a variety of methods known in the art such as solid-phase synthesis, purification of KGF agonists from natural sources, recombinant technology, or a combination of

these methods. See for example, United States Patent Nos. WO 90/08771, and, Dugas and Penney 1981, Merrifield, 1962, Stewart and Young 1969, and the references cited herein.

Purification of a KGF from conditioned medium of a human embryonic fibroblast cell line, the partial amino acid sequencing of a purified KGF, the cloning of the gene, and the expression of the gene in bacterial cells to yield biologically active recombinant KGF have been described. (See for example, WO 90/08771 and Ron et al., J Biol Chem. 268:2984-2988, 1993. See also Rubin, JJ et al, Proc. Natl. Acad. Sci. USA 86:802-806, 1989; Hsu, Y-R, et al, Protein Expression and Purification, 12:189-200, 1998). The cloning of recombinant KGF-2 is described in Igarashi, M., et al, J. Biol. Chem. 273:13230-13235, 1998 and Yamasaki, M. et al, 1996, *supra*.

A KGF agonist, in particular a KGF, can be produced by recombinant methods well known to those skilled in the art. Thus the invention contemplates the use of a nucleotide sequence encoding a KGF agonist, in particular a KGF, and a host cell comprising the nucleotide sequence for the preparation of a KGF agonist.

A "gastrin compound" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially, directly or indirectly, potentiate, induce, mimic, or otherwise enhance the activity of a gastrin or a gastrin/CCK receptor. In particular, a gastrin compound can be used which fully or partially associates and/or activates a gastrin/CCK receptor. A gastrin/CCK receptor includes receptors that associate with a gastrin.

In some applications of the invention, a gastrin compound may be a ligand that associates, binds to, interacts with or stimulates a gastrin/CCK receptor, ("gastrin/CCK receptor ligand). A gastrin compound may be selected that is a peptide or non-peptide small molecule that has a suitable  $IC_{50}$ , for example an  $IC_{50}$  of about  $\sim 0.7$  nM, as measured by methods known in the art (see Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023 describing *in vitro* cell growth assays, and receptor binding assays as described in Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023).

A "gastrin compound" includes native-sequence or synthetic gastrin polypeptides, fragments, analogs (e.g. muteins), derivatives, isoforms, chimeric polypeptides, polypeptides with sequence identity, peptidomimetics, and pharmaceutically acceptable salts thereof, and active metabolites and prodrugs. In particular the term includes the various forms of gastrin, such as gastrin 34 (big gastrin), gastrin 17 (little gastrin), and gastrin 8 (mini gastrin), pentagastrin, tetragastrin and fragments, analogs, and derivatives thereof.

Examples of gastrin compounds that may be used in the present invention include the compounds disclosed in U.S. Patent No. 6,288,301. In some applications of the invention, a gastrin compound may be selected that is a peptide or non-peptide agonist or partial agonist of the gastrin receptor such as A71378 (Lin et al., Am. J. Physiol. 258 (4 Pt 1): G648, 1990). In other applications of the invention, a gastrin compound may be a gastrin/CCK receptor ligand including but not limited to gastrin compounds described herein, or a cholecystokinin (CCK) such as CCK 58, CCK 33, CCK 22, CCK 12 and CCK 8; and the like.

A gastrin compound also includes active analogs, fragments and other modifications, which for example share amino acid sequence identity with an endogenous mammalian gastrin or native-sequence gastrin, for example, share 60%, 70%, 80%, 90%, 95%, 98%, or 99% sequence identity.

Gastrin compounds also include substances that increase the secretion of endogenous gastrins,

cholecystokinins or similarly active peptides from sites of tissue storage. Examples of these are the gastric releasing peptide, omeprazole which inhibits gastric acid secretion and increases plasma gastrin levels, soya bean trypsin inhibitor which increases CCK stimulation, and gastrin releasing peptide, which stimulates gastrin secretion without binding to gastrin receptors.

- 5 Sequences for gastrins including big gastrin-34 (Bonato et al, 1986, Life Science 39:959) and small gastrin-17 (Bentley et al (1966) Nature 209:583) are shown in SEQ ID NOs. 5-9. Big gastrin-34 is essentially an extension of an amino acid sequence at the N-terminal end of small gastrin-17. Big gastrin is cleaved *in vivo* to release gastrin-17. Glp at the N-terminal end is pyroglutamate, which is a naturally cyclized form of glutamate. In various embodiments, where cysteine or lysine are added to a terminus of
- 10 gastrin having a pyroglutamate, the pyroglutamate is replaced with a glutamate, or the pyroglutamate is deleted. Further, each of a gastrin 34 and gastrin-17 can be used having a methionine or a leucine at position 15, as shown in SEQ ID NOs: 5-8 herein.

- A "gastrin compound" includes a modified form of a gastrin. Various gastrins can be modified including but not limited to gastrin 34 (big gastrin), gastrin 17 (little gastrin), and gastrin 8 (mini gastrin),
- 15 pentagastrin, and tetragastrin. Sequences for gastrins including big gastrin-34 (Bonato et al, 1986, Life Science 39:959) and small gastrin-17 (Bentley et al (1966) Nature 209:583) are shown in SEQ ID NOs. 5-9. Modified gastrin compounds for use in the present invention comprise the modified gastrin compounds described in PCT/CA03/01778, US Serial No. 10/719,450 and U.S. Application Serial No. 60/519,933 incorporated in their entirety by reference. A modified gastrin compound can have an extended activity
- 20 upon administration to a subject in comparison to a native-sequence gastrin. They may have a longer half-life in the circulation of a subject.

- A modified gastrin compound can contain a minimal gastrin component which comprises at least amino acids at positions 29-34 of SEQ ID NO:5 or at positions 12-17 of SEQ ID NO:6. These are located at the carboxy terminus of gastrins that occur in circulation, and in various gastrin compounds contemplated
- 25 herein additional amino acids, for example from gastrin, can be present.

- A group of modified gastrin compounds include compounds having an amino acid sequence comprising from the amino terminus  $Z-Y_m-X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6$ , wherein  $AA_1$  is Tyr or Phe,  $AA_2$  is Gly, Ala, or Ser,  $AA_3$  is Trp, Val, or Ile,  $AA_4$  is Met or Leu,  $AA_5$  is Asp or Glu, and  $AA_6$  is Phe or Tyr which can be amidated; Z is the sequence of a polymer or a protein (e.g. a serum protein such as human serum albumin);  $Y_m$  is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine, glycine, and/or alanine, and X is any consecutive portion of residues
- 30 1-28 of SEQ ID NO: 5, residues 1-28 of SEQ ID NO: 6; residues 1-11 of SEQ ID NO: 7; or residues 1-11 of SEQ ID NO: 8, providing that the gastrin compound binds a gastrin/CCK receptor. Generally, m is 0 to about 20 residues.

- $Y_m$  can be an amino acid sequence comprising m residues having glycine alternating with alanine, for example, [Gly-Ala]<sub>m</sub>, or having random sequence of glycine and alanine. The gastrin compound further can have a cysteine residue at the amino terminus of Y, when m is greater than 1, or at the amino terminus of X, when m is 0. The gastrin compound can further comprise a bifunctional crosslinking agent for linkage to Z. In general, m is 0 to about 20 residues. In certain embodiments, m is 0, and the compound is  $X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6$  further comprising a bifunctional cross-linking agent for linkage to Z.
- 40

The X in certain embodiments is selected from the group of sequences: position 1 to position 11 of SEQ ID NO: 5; position 1 to position 11 of SEQ ID NO: 6; position 2 to position 11 of SEQ ID NO: 7; and position 2 to position 11 of SEQ ID NO: 8. The gastrin compound in which Z is a protein can be recombinantly produced.

- 5 In preferred embodiments, X is one or more amino acid residues from position 18 to position 28 of SEQ ID NO: 5 or 6. Therefore, the gastrin compounds by virtue of the presence of X, can have any of gastrin sequences from positions 18-28, 19-28, 20-28, 21-28, etc. The gastrin compound optionally contains an amino acid spacer of length m, and m is 0 to about 20 residues.

- 10 A modified gastrin compound of the formula  $X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6$  where there is no spacer and m is 0, may further comprise a bifunctional cross-linking agent for linkage to Z, where Z further comprises a non-proteinaceous polymer.

In some embodiments of modified gastrin compounds described herein, the gastrin component contains at least amino acid residues 29-34 of SEQ ID NO: 5 or 6, and it is associated with a polymer, a lipid or a carbohydrate.

- 15 A polymer may be a synthetic or naturally occurring polymer. The term polymer includes a protein polymer of amino acids, and is not limited to a synthetic polymer. The polymer may be a polyethylene glycol (PEG) or a dextran. When the polymer is a protein, in various embodiments it can be a serum protein, for example, a serum albumin, for example, human serum albumin.

- 20 A modified gastrin compound can be based on SEQ ID NO: 5 or 6 or "big" gastrin-34 and have a residue at position 32 which is a methionine or a leucine, respectively.

- Another preferred modified gastrin compound comprises a structure  $Z-Y_m-X$ , wherein Z is Cys or Lys,  $Y_m$  is an optional spacer region comprising m amino acid residues of a small neutral amino acid, and X is at least six amino acid residues comprising at least positions 12-17 of gastrin-17 (SEQ ID NO: 7 or 8) or at least positions 29-34 of gastrin-34 (SEQ ID NO: 5 or 6). This modified gastrin compound can further  
25 comprise a bifunctional cross-linking agent wherein one reactive portion of the cross-linking agent is covalently linked to Z, and the other reactive portion is covalently linked to a polymer or protein.

- A modified gastrin can be a gastrin derivative or analog comprising a minimal sequence of 6 amino acids (from the C-terminal end), and further having addition of a reactive group capable of undergoing an addition reaction. Thus, a modified gastrin compound may further comprise at least one reactive amino acid  
30 such as a cysteine or lysine residue. The reactive amino acid may be added or substituted at the N-terminal end. The addition of the reactive amino acid can be at a terminal region and a spacer region can optionally precede the added reactive residue. For example, the spacer can be synthesized biologically as part of, or can be chemically attached to the gastrin amino acid sequence, forming a structure which has a gastrin sequence-spacer-cysteine/lysine. The spacer may be a string of several amino acids such as alanine or glycine.

- 35 In an embodiment, a modified gastrin compound  $AA_1-AA_2-AA_3-AA_4-AA_5-AA_6$  is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe.

A modified gastrin compound can comprise a moiety that is a gastrin compound associated with a larger molecule such as a polymer, either non-covalently, or as a covalent conjugate, or as a fusion protein to another peptidic compound having an amino acid sequence. The modified gastrin compounds can have a



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longer half-life in circulation in a subject animal or patient, and/or maintain higher concentrations *in vivo* of the gastrin compounds for an extended period of time compared to the native forms of gastrin.

In an embodiment, a modified gastrin compound comprises a gastrin compound bound to a comparatively larger structure or a plurality of structures in the blood and still retains the ability to bind target proteins, i.e., a gastrin/CCK receptor. Generally, a gastrin compound is attached to a carrier protein which can result in a longer-term of drug efficacy. Alternatively a gastrin compound can be conjugated to a polymeric carrier such as a polyethylene glycol (PEG) or a dextran to achieve similar objectives

In embodiments of the invention, chemical modification of a gastrin compound is used to provide compounds that react covalently or non-covalently to carrier proteins or polymeric carriers, either *in vitro* (*ex vivo*) or *in vivo*. In a particular embodiment, the non-covalent interaction is electrostatic or hydrophobic. In certain embodiments, conjugation of a gastrin compound to the carrier is carried out prior to injection. In other embodiments, the gastrin compound is modified in such a manner that when injected, will have an enhanced affinity to the carrier in the bloodstream. In another embodiment, long acting gastrin compounds are obtained via chemical modification with no requirement for a carrier protein either *in vivo* or *ex vivo*.

In certain embodiments, the carrier protein is a plasma protein. In related embodiments, the plasma protein is an albumin or an immunoglobulin or components of an immunoglobulin. The immunoglobulin or components of the immunoglobulin can be modified or portions deleted prior to conjugation. In certain embodiments, the polymeric carrier is polyethylene glycol or dextran. For instance, activated PEG can be attached to a gastrin compound via an amino group in the gastrin compound (Vernonese, FM. Biomaterials 22(2001)-405-417).

In other embodiments, the gastrin compound which is a sequence of amino acids, is genetically fused with a carrier protein, which is also a sequence of amino acids, prior to injection, using standard recombinant genetic techniques. A gastrin compound can be fused recombinantly to a carrier protein with or without a linker/spacer, for example, comprising a sequence of small neutral uncharged amino acids. A nucleic acid encoding a gastrin compound can be recombinantly fused or synthesized directly as a fusion to portions or the whole of the carrier protein, and the nucleic acid construct or fusion protein can encode or incorporate a number of additional amino acids to act as a spacer between the two proteins. Recombinant fusion proteins can be expressed in a host cell. Modifications to the sequence of gastrin compound polypeptide can be introduced during construction of the fusion protein if necessary.

In one embodiment, the gastrin compound is modified to introduce a reactive group such as those present on an amino acid such as a lysine or cysteine so that the reactive group upon further contacting another compound such as a carrier protein or carrier non-proteinaceous polymer, can form covalent interactions with the carrier proteins or polymers. For instance, a reactive thiol group can be added to a gastrin compound through an amino group on lysine, for example, using succinimidyl 3-(2-pyridyldithio)propionate (SPDP) followed by reduction with DTT to release the active thiol group ("Protein thiolation and reversible protein-protein conjugation. N-Succinimidyl 3-(2-pyridyldithio)propionate, a new heterobifunctional reagent." Carlsson J, Drevin H, Axen R. Biochem J 173, 723-737 (1978)). Further, the bifunctional group can also be added after the cysteine or lysine has been added, so that one reactive end of the crosslinking agent will react with cysteine/lysine while the other reactive end at the other end is left exposed or is conjugated to a carrier.

Thiols can be also incorporated at carboxylic acid groups by EDAC-mediated reaction with cystamine, followed by reduction of the disulfide with DTT. ("Introduction of sulfhydryl groups into proteins at carboxyl sites." Lin CM, Mihal KA, Krueger RJ. *Biochim Biophys Acta* 1038, 382-385 (1990). In a non-limiting example, reaction of an amino group on the lysine residue in a gastrin compound with

5 succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate ("Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using N-hydroxysuccinimide ester of N-(4-carboxycyclohexylmethyl)-maleimide." Yoshitake S, Yamada Y, Ishikawa E, Masseyeff R. *Eur J Biochem* 101, 395-399 (1979)) introduces a thiol reactive group at amino sites of a gastrin compound that can subsequently react with cysteine residues of the carrier protein or free thiol group on the activated polymer.

10 A gastrin compound-carrier complex can include additional modular components including a spacer arm or element or other component that can facilitate preparation or isolation of the gastrin compound-carrier complex or enhance or maintain the functional activity of the gastrin compound. The spacer arm can be one or more amino acids, peptide, a peptidomimetic, or a small organic molecule, and can comprise homobifunctional or heterobifunctional crosslinking agents or chitin oligomers or polyethylene glycol or

15 related polymers.

In another embodiment, the carrier and gastrin compound can be covalently crosslinked with or without a spacer arm. Examples of non-spacer arms (zero-length crosslinkers) include EDC. Homobifunctional crosslinkers that generate a spacer arm can be for instance disuccinimidyl suberate and heterobifunctional crosslinkers that generate a spacer arm can be for instance 2-iminothiolane, succinimidyl

20 6-[3-(2-pyridyldithio)propionamido] hexanoate (LC-SPDP) and 4-(N-maleimido methyl)cyclohexane-1-carboxylate (SMCC).

In various embodiments, a gastrin compound is associated with a larger carrier moiety such as a polymer, for example a protein. As the association may be covalent or non-covalent, the protein may be considered to be a carrier protein. Classes of carrier proteins can possess the properties of being non-

25 antigenic, i.e., are native human proteins, and are being capable of sustained maintenance in circulation. An ideal carrier protein is one normally found in the human circulatory system.

Gastrin compounds may be prepared using conventional processes. For example, small forms of gastrin such as gastrin 17 are economically prepared by peptide synthesis, and the synthetic peptides are commercially available. Synthetic human gastrin 17, and derivatives such as human gastrin 17 having

30 leucine substituted for methionine at position 15 are also available from Bachem AG, Bubendorf, Switzerland, and from ResearchPlus.

In particular, gastrin compounds may be synthesized by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, *J. Am. Chem. Assoc.* 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, *Methods of Organic Chemistry*, ed.

35 B. Wansch, Vol. 15 I and II, Thieme, Stuttgart). The synthesis may be performed using manual procedures or by automation. Automated synthesis may be carried out, for example, using an Applied Biosystems 431A peptide synthesizer (Perkin Elmer). Gastrin compounds may also be obtained from commercial sources. For example, synthetic human gastrin 17 with methionine or leucine at position 15 are available from Bachem AG, Bubendorf, (Switzerland), and from Research Plus Inc (New Jersey, USA).

Gastrin compounds can be prepared by recombinant methods well known to those skilled in the art. Thus the invention contemplates the use of a nucleotide sequence encoding a gastrin compound, and a host cell comprising the nucleotide sequence for the preparation of a gastrin compound.

5 "Host cells" comprising a nucleotide sequence of a KGF agonist or gastrin compound include a wide variety of prokaryotic and eukaryotic host cells. For example, the polypeptides may be expressed in bacterial cells such as *E. coli*, *Bacillus*, or *Streptomyces*, insect cells (using baculovirus), yeast cells, or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991). A host cell may also be chosen which modulates the expression of an inserted nucleotide sequence, or modifies (e.g. glycosylation or phosphorylation) and processes (e.g., cleaves) the polypeptide in a desired fashion. Host systems or cell lines may be selected which have specific and characteristic mechanisms for post-translational processing and modification of proteins. For long-term high-yield stable expression of the protein, cell lines and host systems which stably express the gene product may be engineered.

15 "Regulatory element" refers to a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating recombinant constructs encoding a KGF agonist or gastrin compound. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrec99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTL pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). As defined herein 20 "operably linked" means that an isolated polynucleotide and a regulatory element are situated within a vector or cell in such a way that the polypeptide is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/regulatory element sequence.

25 "Condition(s)" and/or "disease(s)" refer to one or more pathological symptoms or syndromes for which either or both a KGF agonist or a gastrin compound provide a therapeutic effect. The condition or disease may require reduction of blood glucose levels, inhibition of gastric acid secretion, inhibition of apoptosis of  $\beta$ -cells, stimulation of proliferation or differentiation of  $\beta$ -cells, and reduction of body weight. Examples of conditions and diseases include but are not limited to dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable 30 bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, obesity, diabetic complications as well as symptoms of other diseases in which tissue is damaged due to elevated glucose levels, including Alzheimer's Disease, Parkinson's Disease, and other age-related, tissue-degenerative 35 diseases, as well as the arterogenic effects of elevated leptin, for example in patients with impaired glucose tolerance and obese non-diabetic patients.

The term, "diabetes" as used herein means any manifested symptoms of diabetes in any mammal including experimental animal models, and including human forms such as type I and type II diabetes, early stage diabetes, and a pre-diabetic condition characterized by mildly decreased insulin or mildly elevated 40 blood glucose levels. A "pre-diabetic condition" describes a subject demonstrating a symptom in terms of

insulin or glucose level, and/or demonstrating a susceptibility to diabetes or a related condition due to family history, genetic predisposition, or obesity in the case of type II diabetes, and includes a subject who has previously had diabetes or a related condition and is subject to risk of recurrence.

5 "Insulinotropic activity" refers to an ability of a substance to stimulate insulin secretion in response to elevated glucose levels to produce or increase glucose uptake by cells and decreased serum glucose or blood glucose levels. Methods known in the art can be employed to assay for insulinotropic activity. For example, *in vitro* and *in vivo* methods may be used that measure KGF receptor binding activity or gastrin receptor binding activity, receptor activation (see the methods described in EP 619,322 to Gelfand et al and US Patent No. 5,120,712), and insulin or C-peptide levels. Compounds, compositions or conjugates  
10 described herein have insulinotropic activity if islet cells secrete insulin in the presence of the compounds, compositions, or conjugates above background levels or levels in the absence of the compounds, compositions, or conjugates.

A condition or disease includes applications requiring stimulation of epithelial cells, in particular keratinocytes. The conditions may be characterized by damage or deficiencies in these particular cell types.

15 Recombinant KGF has been demonstrated to increase re-epithelialization and increased thickness of the epithelium when topically applied to wounds surgically induced in the rabbit ear or in the porcine skin. (Pierce et al., J. Exp. Med. 179:831-840, 1994); and Staiano-Coico et al., J. Exp. Med. 178:865-878, 1993. Therefore, KGF agonists in combination with a gastrin compound can be used as wound healing agents for burn wounds or to stimulate transplanted corneal tissue.

20 The compounds, compositions and conjugates of the invention may be used to promote and/or enhance soft-tissue growth and regeneration. In particular they may be used to stimulate proliferation and differentiation of adnexal structures such as hair follicles, sweat glands, and sebaceous glands for example, to regenerate epidermis and dermis in patients with burns and other partial and full thickness injuries. Therefore, conditions and diseases contemplated herein also include but are not limited to epidermolysis  
25 bullosa, chemotherapy induced alopecia, male-pattern baldness, gastric ulcers, duodenal ulcers, inflammatory bowel diseases (e.g. Crohn's disease and ulcerative colitis), gut toxicity in radiation and chemotherapy, hyaline membrane disease of premature infants, inhalation injuries, emphysema, hepatic cirrhosis (e.g. secondary to viral hepatitis and chronic alcohol ingestion), fulminant liver failure, acute viral hepatitis, toxic insults to the liver caused by acetaminophen, halothane, carbon tetrachloride, and other  
30 toxins, and conditions requiring production of mucus in the gastrointestinal tract.

The compounds, compositions and conjugates of the invention may be used to stimulate growth of gastrointestinal mucosa and promote healing after intestinal injury (see Playford, R.J. J. Pathol. 184:316-322, 1998). Therefore, they may have particular application in the treatment of mucositis (loss of gut epithelial tissue after chemotherapy or radiation therapy for cancer).

35 "Islet neogenesis" means formation of new beta cells by proliferation and differentiation, which may or may not have the characteristics of stem cells which have the ability to reproduce in an unlimited manner.

#### KGF Agonist and Gastrin Compound

40 The invention is related to compositions, conjugates, and methods that utilize a KGF agonist and a gastrin compound, in particular to provide beneficial effects. The compositions, conjugates and methods of

the invention provide enhanced beneficial effects, in particular sustained beneficial effects relative to a KGF agonist and/or a gastrin compound alone. In embodiments of the invention, the beneficial effects are additive or synergistic.

In an embodiment, where the disease or condition is diabetes, sustained beneficial effects of a composition, combination treatment, or conjugate of the invention may manifest as one or more of the following:

- An increase in pancreatic insulin levels relative to the levels measured in the absence of the active compounds or for each compound alone after administration to a subject with symptoms of diabetes. Preferably the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% increase in pancreatic insulin levels in a subject.
- A reduction of an absence of symptoms of islet inflammation after administration to a subject with symptoms of diabetes.
- A decrease in blood glucose levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds induce at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels. Most preferably, the compounds yield blood glucose levels about or close to the levels common in a normal subject.
- An improvement in glucose tolerance. In particular, at least about a 10-90% improvement in glucose tolerance.
- An increase in C-peptide levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% increase in C-peptide levels.
- Maintenance of blood glucose levels at about normal for a prolonged period of time, in particular, for at least 1 week, 4 weeks, 16 weeks, 52 weeks, or 78 weeks.
- A reduction, prevention, or slowing of the rate of disease progression in a subject with diabetes.
- A reduction or prevention of the development of severe hyperglycemia and ketoacidosis with symptoms of diabetes.
- An increase in survival in a subject with symptoms of diabetes.
- A decrease in requirement for insulin injection/intake by at least 10-90%, 10-80%, 10-70%, 10-60%, 10-50%, 10-40%, 10-30%, or 10-20%.

One or more of these beneficial effects can be demonstrated in a diabetic subject or disease model, for example a non-obese (NOD) mouse with symptoms of diabetes.

A gastrin compound is selected for particular embodiments in the present invention and to provide a specific beneficial effect(s) based on characteristics including the ability to augment the activity of a KGF agonist and/or increase the physical or chemical stability of a KGF agonist. A gastrin compound can also be

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selected based on its ability to stimulate proliferation/differentiation of beta cells, its *in vivo* half-life, or its insulinotropic activity.

In an embodiment of the invention, the gastrin compound is gastrin 17 and analogs and derivatives thereof. In a particular embodiment, the gastrin compound is synthetic human gastrin I having 17 amino acid  
5 residues with a Leu residue at amino acid position 15.

A KGF agonist may be selected for particular applications in the present invention based on one or more of the following characteristics: ability to bind to the KGF receptor, ability to initiate a signal transduction pathway resulting in proliferation and/or differentiation of beta cells or insulinotropic activity; ability to reduce glucose levels, insulinotropic activity; stimulation of beta cell proliferation/differentiation;  
10 and/or, an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans using conventional methods (see for example, the method described in US 2003/0144206)

In an embodiment of the invention the KGF agonist is a truncated polypeptide, or an analog or derivative thereof. The sequences of these truncated KGF agonists are represented in US 20030109439, US 5,677,278, WO 3095637, and US 6677301.

15 In another embodiment of the invention, the KGF agonist is an analog of KGF or KGF-2 which has less than 10 amino acid residues that are different from those in KGF or KGF-2, less than 5 amino acid residues that are different from those in KGF, or less than 3 amino acid residues that are different from those in KGF or KGF-2, preferably only one amino acid residue that is different from sequence of a KGF or KGF-2.

20 KGF agonists that may have specific utility in the present invention include polypeptides where one or more amino acids have been added to the N-terminus and/or C-terminus of KGF or KGF-2. Preferably, about 1-150 amino acids may be added to the N-terminus and/or from about 1-150 amino acids may be added to the C-terminus.

In another embodiment of the invention at least one amino acid of a KGF agonist has at least one  
25 substituent attached directly or indirectly (e.g. via a spacer such as  $\gamma$ -Glu or  $\beta$ -Ala). A substituent is generally selected to make the profile of action of the parent KGF agonist more protracted, make the KGF agonists more metabolically and physically stable, and/or increase solubility of the KGF agonist. An example of a particular substituent is a lipophilic substituent including but not limited to an alkyl group, a group which has an  $\alpha$ -carboxylic acid group, an acyl group of a straight-chain or branched fatty acid or alkane such as  
30 tetradecanoyl, hexadecanoyl.

In embodiments of the invention, the KGF agonist is selected from the group consisting of KGF-1 and KGF-2.

#### Compositions

The invention contemplates a composition, preferably a pharmaceutical composition, comprising a  
35 KGF agonist and a gastrin compound, in particular a gastrin/CCK receptor ligand. A pharmaceutical composition may optionally comprise a pharmaceutically acceptable carrier, excipient, or vehicle. In an aspect, the composition provides beneficial effects relative to each compound alone. In another aspect, the invention contemplates a pharmaceutical composition comprising a KGF agonist and a gastrin compound, in

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particular a gastrin/CCK receptor ligand, which provides beneficial effects, preferably sustained beneficial effects, following treatment.

A KGF agonist and a gastrin compound in a composition of the invention may be in a ratio selected to augment the activity of the agonist and/or gastrin compound to provide a beneficial effect.

- 5       The invention also provides a pharmaceutical composition in separate containers and intended for simultaneous or sequential administration to provide beneficial effects, comprising a KGF agonist and a gastrin compound, both optionally together with pharmaceutically acceptable carriers, excipients, or vehicles.

- 10       Pharmaceutical compositions of the invention can be selected that have statistically significant sustained beneficial effects, preferably sustained beneficial effects, compared with a KGF agonist or a gastrin compound alone.

- 15       In an embodiment, a pharmaceutical composition with statistically significant beneficial effects is provided comprising a KGF agonist selected from the group consisting of KGF and KGF-2, and a gastrin compound selected from the group consisting of gastrin 17 and analogs and derivatives thereof, preferably synthetic human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15.

In an embodiment, a pharmaceutical composition with statistically significant beneficial effects is provided comprising KGF and gastrin-17(leu).

In an embodiment, the invention comprises pharmaceutically acceptable salts of a KGF agonist and/or pharmaceutically acceptable salts of a gastrin compound.

- 20       In another embodiment, a pharmaceutical composition is provided which has been adapted for administration to a subject to provide sustained beneficial effects to treat a condition or disease, preferably diabetes. In a preferred embodiment, it is in a form such that administration to a subject results in blood glucose levels that are about normal that persist in the subject for a prolonged period of time after cessation of treatment.

- 25       In an embodiment, a composition comprising a KGF agonist and a gastrin compound have greater sustained insulinotropic activity following treatment compared with the activity of a KGF agonist or gastrin compound alone or greater than KGF alone.

#### Conjugates

- 30       This invention provides a conjugate comprising a KGF agonist linked to a gastrin compound wherein the linkage is, for example, via an amino or carboxyl group. The invention also relates to isolated covalent conjugates of the invention, and compositions comprising covalent conjugates of the invention.

A KGF agonist may be conjugated to a species via an ester bond between a OH and a COOH of a gastrin compound.

- 35       Conjugates of a KGF agonist and a gastrin compound may be conjugated or linked with an intermediate spacer or linker. A suitable spacer or linker may be a mono- or disaccharide, an amino acid, a sulfate, a succinate, an acetate, or an oligomeric polymeric spacer or linker comprising one or more of such moieties.

The invention also provides methods of preparing the above covalent conjugates that result in conjugates with improved pharmacokinetic properties, biological activity, and beneficial effects. The

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methods comprise incubating or reacting the KGF agonist with the gastrin compound under conditions that allow formation of a covalent linkage between the two compounds.

5 The invention therefore contemplates a process for preparing a covalent conjugate, comprising a KGF agonist covalently bonded or linked to a gastrin compound, the process comprising: incubating or reacting the KGF agonist with a gastrin compound under conditions and at a pH and for a time sufficient for formation of a covalent bond or linkage between the KGF agonist and gastrin compound; and isolating the covalent conjugate.

The above process for preparing a conjugate comprising a KGF agonist and a gastrin compound can provide a conjugate with a substantial amount of a KGF agonist covalently linked to a gastrin compound.

10 N-terminal or C-terminal fusion proteins or chimeric proteins, comprising a KGF agonist conjugated with a gastrin compound, optionally with a spacer or linker, may also be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal sequence of a KGF agonist and the sequence of a gastrin compound.

The invention also provides a conjugate prepared by a process described herein.

15 The invention also relates to pharmaceutical formulations comprising conjugates of the invention and a pharmaceutically acceptable carrier, excipient, or vehicle.

The invention further relates to a pharmaceutical formulation of a substantially pure covalent conjugate comprising a KGF agonist covalently linked to a gastrin compound which provides beneficial effects, preferably sustained beneficial effects, compared to the KGF agonist alone.

20 In an embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising a KGF agonist covalently linked without an intermediate spacer or linker to a gastrin compound.

#### Applications

25 The invention also contemplates the use of a composition of the invention or combination treatment of the invention for preventing, and/or ameliorating disease severity, disease symptoms, and/or periodicity of recurrence of a disease or condition described herein. The invention also relates to the prevention and treatment, in mammals, of conditions or diseases using the compositions, combination treatments, and conjugates of the invention.

30 In an aspect the invention provides a combination treatment for preventing and/or treating a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one KGF agonist and at least one gastrin compound to produce beneficial effects, preferably sustained beneficial effects.

35 The present invention includes combination treatments providing additive or synergistic activity, delivering an additive or synergistically effective amount, or an amount to provide a therapeutically effective amount of a KGF agonist and a gastrin compound, or a conjugate or composition of the invention. Therefore, pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in a synergistically effective amount or a therapeutically effective amount.

40 An embodiment of the present invention provides improved methods and compositions for use of a KGF and a gastrin compound to treat diabetes. The present invention in one embodiment provides a combination of gastrin with KGF to achieve greater efficacy, potency, and utility than achieved with KGF



alone, resulting in an improved therapeutic ratio for combination. The greater efficacy can be shown by improving glucose tolerance in severe diabetes with treatment resulting in prolonged improvement of blood glucose after ceasing treatment (Example 6) and also in recent onset diabetes (Example 7).

5 Treatment with a combination of gastrin and KGF can provide a reduction in blood glucose that is greater than observed after treatment with KGF alone, and the reduction can be sustained for prolonged periods after ceasing treatment in severe diabetes (Example 5) and recent onset diabetes (Example 7).

In combination with gastrin, systemic administration of KGF can have greater potency than when KGF is administered alone. An improvement in glucose tolerance is observed with the KGF/gastrin combinations using lower doses of KGF that are not effective when administered alone, i.e. doses below 1  
10 mg/kg body weight.

Greater efficacy and potency of the combination of gastrin and KGF improves the therapeutic ratio of treatment, since use of the lower doses of KGF reduces untoward side effects and toxicity resulting from KGF induced proliferation of other organs such as the liver. The combination also enhances utility improving long-standing diabetes even when treatment is begun long after the completion of  $\beta$  cell  
15 destruction (see Example 1 and 2). The gastrin/KGF combination can be effective in reducing blood glucose with treatment initiated as long as 3-4 weeks after streptozotocin-induced  $\beta$  cell destruction.

Improvement in glucose tolerance after treatment with the gastrin/KGF combination results from  $\beta$  cell regeneration and concomitant increased  $\beta$  cell mass (Example 4). Histological analysis can show treatment with gastrin/KGF stimulates  $\beta$  cell regeneration with an increase in the  $\beta$  cell mass as measured  
20 morphometrically. This can be confirmed biochemically by an increase in pancreatic insulin content. The increased  $\beta$  cell mass can also result in increased secretion of insulin into the bloodstream which can be shown by increased circulating C peptide in plasma (Example 3).

The invention also relates to a method of treatment comprising administering a therapeutically effective amount of at least one KGF agonist in combination with the administration of at least one gastrin  
25 compound which upon administration to a subject with symptoms of diabetes produces beneficial effects, preferably sustained beneficial effects, manifested as reduced blood glucose levels and/or increased pancreatic insulin.

In an aspect of the invention therapeutically effective amounts of a KGF agonist and a gastrin compound are combined prior to administration to a subject. In an embodiment, therapeutically effective  
30 amounts of a KGF agonist and a gastrin compound are mixed at a physiologically acceptable pH.

In a further embodiment, the invention provides a method for preventing and/or treating Type I or Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a KGF agonist and a gastrin compound.

In a further embodiment, the invention provides a method for ameliorating progression of disease  
35 or obtaining a less severe stage of disease in a person suffering from Type I or Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a KGF agonist and a gastrin compound.

The invention relates to a method of delaying the progression of impaired glucose tolerance or non-insulin requiring Type II diabetes to insulin requiring Type II diabetes comprising administering a

therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a KGF agonist and a gastrin compound.

The invention also relates to a method of increasing the insulin synthesis capability of a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a KGF agonist and a gastrin compound.

An approach to  $\beta$  cell replacement which avoids cell transplantation is to stimulate  $\beta$  cell regeneration. Although early studies suggested that the  $\beta$  cell has limited capacity for regeneration, it has been increasingly realized that the insulin secreting  $\beta$  cells of the pancreas comprise a dynamic cell population. The mass of  $\beta$  cells can expand through proliferation of existing  $\beta$  cells ( $\beta$  cell replication). During pregnancy, prolactin, growth hormone (Holstad, M. et al., J. Endocrinol. 163:229-234), and placental lactogen (Nielsen, J.H. et al, J. Mol. Med. 77:62-66, 1999) stimulate the proliferation of  $\beta$  cells to increase  $\beta$  cell mass. However, this expanded mass is dependent on continued hormonal stimulation. After parturition, the expanded  $\beta$  cell mass decreases to non-pregnant levels along with the decrease in prolactin and placental lactogen ((Logothetopoulos, J. (1972) in Handbook of Physiology (Am. Physiol. Soc., Washington, DC), Section 7 Chapter 3, pp. 67-76). Thus, an important aspect in evaluating  $\beta$  cell regeneration in response to administration of growth factors is whether the expanded  $\beta$  cell mass persists for a significant time after the cessation of treatment with growth factors.

In an embodiment, the invention provides a method for stimulating beta cell proliferation in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a KGF agonist and a gastrin compound.

In another embodiment, the invention provides a method for increasing the number and/or size of beta cells in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention or administering in combination a KGF agonist and a gastrin compound.

A method for treating diabetes mellitus in an individual in need thereof includes administering to the individual a composition that provides both a gastrin/CCK receptor ligand and a KGF receptor ligand, in doses sufficient to effect differentiation of pancreatic islet precursor cells to mature insulin-secreting cells. A method for treating insulin dependent diabetes, especially Type I or juvenile diabetes mellitus, comprises administering, preferably systemically, a differentiation regenerative amount of both a gastrin/CCK receptor ligand and a KGF receptor ligand, to a diabetic mammal, to stimulate islet neogenesis to increase the number of functional glucose responsive insulin secreting  $\beta$  cells in the pancreas. The combination of gastrin and KGF receptor ligand would result in significant enhancement of the islet neogenesis response over that observed with either of the gastrin or the KGF receptor ligand alone. A preferred gastrin/CCK receptor ligand is gastrin, and a preferred KGF receptor ligand is KGF (FGF-7) or KGF-2 (FGF-10).

Another embodiment is a method comprising treating cells, or treating explanted pancreatic tissue of a mammal with a gastrin/CCK receptor ligand and KGF receptor ligand and introducing the treated cells or pancreatic tissue to the mammal. In this embodiment, the preferred gastrin/CCK receptor ligand is gastrin and the preferred KGF receptor ligand is KGF (FGF-7) or KGF-2(FGF-10).

In another embodiment, the invention provides a method for gastrin/CCK receptor ligand stimulation comprising providing a chimeric insulin promoter-gastrin fusion gene to pancreatic cells and

expressing the gene. In a further embodiment, a method of KGF receptor ligand stimulation is provided, comprising expression of a KGF receptor ligand gene transgenically introduced into a mammal, for example, a KGF receptor ligand is KGF (FGF-7) similarly provided as in US Patent No. 5,885,956.

5 The invention provides a method for treating diabetes mellitus by administering a composition comprising both a gastrin/CCK receptor ligand, e.g. gastrin, and a KGF receptor ligand, e.g. KGF, KGF-2, in an amount sufficient to effect differentiation of pancreatic islet precursor cells to mature insulin-secreting cells. Both ligands in the composition can be administered systemically. Alternatively, one or both ligands can be expressed in situ by cells provided with a nucleic acid fusion construct in an expression vector. The fusion construct typically includes a preprogastrin peptide precursor coding sequence, and also a coding  
10 sequence for a KGF receptor ligand.

Prolonged efficacious islet cell neogenesis is achieved following administration of both a gastrin/CCK receptor ligand, such as gastrin, and a growth factor ligand of the KGF tyrosine kinase receptor (TKR) family, such as KGF.

15 Regenerative differentiation of pluripotent pancreatic precursor cells, for example, pancreatic ductal cells, into mature insulin-secreting cells is obtained with the provided compositions, conjugates, and methods for treatment of diabetes mellitus, particularly juvenile onset diabetes, and by therapeutic administration of this combination of factors or compositions which are provided for systemic administration, or for in situ expression within the pancreas.

20 The invention provides methods for treating cells, preferably cells in culture using a KGF agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. The invention also provides cell based treatment methods using a KGF agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. See PCT/CA03/33595 for a description of general culture and cell based treatment methods.

25 In an aspect, the invention provides a method of treating a condition or disease comprising administering a KGF agonist and a gastrin compound, a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce a beneficial effect, preferably a sustained beneficial effect.

30 A method for treating a subject with a condition or disease described herein comprises contacting *ex vivo* a plurality of cells with a KGF agonist and a gastrin compound, or a composition or conjugate of the invention of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

35 In embodiments of the aforementioned cell based therapeutic methods the cells are pancreatic ductal cells and the amount of compounds/composition/conjugate used in the method is generally effective to increase the amount of insulin secreting cells in the subject. The cells may be autologous (i.e. from the same subject), or may be from another individual of the same species, or from a different species.

The invention also relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with a KGF agonist and a gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

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The invention also relates to a method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective amount of a KGF agonist and a gastrin compound or a composition or conjugate of the invention. The compounds, composition or conjugates may be administered to a subject before, during, or after stem cells are implanted in the subject. The stem cells  
5 may be obtained from pancreatic islets, umbilical cords, embryos, or stem cell lines. The method may additionally comprise administering an immunosuppressive agent.

The invention contemplates a method of expanding a functional beta cell mass of pancreatic islet transplants in a diabetic patient, the method comprising administering to the patient a therapeutically effective amount of a KGF agonist and a gastrin compound, or a composition or conjugate of the invention.

10 The invention also contemplates a method for treating diabetes in a subject comprising transplanting a pancreatic islet preparation into the subject and administering a therapeutically effective amount of a KGF agonist and a gastrin compound, or a composition or conjugate of the invention.

The methods of the invention may further comprise measuring one or more of the following markers: blood glucose, serum glucose, blood glycosylated haemoglobin, pancreatic beta cell mass, serum  
15 insulin, pancreatic insulin levels, morphometrically determined beta cell mass, amount of insulin secreting cells, and glucose responsiveness of insulin secreting cells.

The invention also contemplates the use of a composition comprising a combination of at least one KGF agonist and at least one gastrin compound for the preparation of a medicament providing beneficial effects, preferably sustained beneficial effects in treating a condition or disease.

20 In an embodiment, the invention relates to the use of a therapeutically effective amount of at least one KGF agonist, and at least one gastrin compound for preparation of a medicament for providing beneficial effects, preferably sustained beneficial effects, in treating a condition or disease.

In an embodiment the invention provides the use of a KGF agonist and a gastrin compound for the preparation of a medicament for increase (preferably sustained increase) of the number and/or size of beta  
25 cells in a subject after treatment.

In another embodiment the invention provides the use of KGF agonist and a gastrin compound for the preparation of a medicament for stimulation (preferably sustained stimulation) of beta cell proliferation after treatment.

30 In a still further embodiment the invention provides the use of KGF and gastrin for the preparation of a medicament for treatment of Type I or Type II diabetes.

The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for beneficial effects, preferably sustained beneficial effects, in the treatment of diseases and conditions.

35 Therapeutic efficacy and toxicity of compositions, conjugates and methods of the invention may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals such as by calculating a statistical parameter such as the  $ED_{50}$  (the dose that is therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The therapeutic index is the dose ratio of therapeutic to toxic effects and it can be expressed as the  $ED_{50}/LD_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

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The present invention also includes methods of using the compositions of the invention in combination with one or more additional therapeutic agents including without limitation immunosuppressive agents (e.g. rapamycin, cyclosporine, ISAtx247, and FK506), antiobesity agents, antidiabetic agents, appetite regulating drugs, antihypertensive agents, agents for the treatment and/or prevention of complications  
5 resulting from or associated with a condition or disease, in particular diabetes and obesity, anti-nausea, anti-headache medications, and general medications that treat or prevent side effects

#### Administration

A gastrin compound in combination with a KGF agonist, conjugates, and compositions of the present invention can be administered by any means that produce contact of the active agent(s) with the  
10 agent's sites of action in the body of a subject or patient. The active ingredients can be administered simultaneously or sequentially and in any order at different points in time, to provide the desired beneficial effects. The compounds, conjugates and compositions can be formulated for sustained release, for delivery locally or systemically. It lies within the capability of a skilled physician or veterinarian to select a form and route of administration that optimizes the effects of the compositions, conjugates, and treatments of the  
15 present invention.

Modes of parenteral administration include, but are not limited to, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g. oral mucosa, rectal and intestinal mucosa, etc.), and may be administered  
20 together with other biologically active agents. A preferred route of administration is systemic, for example, by subcutaneous injection. For parenteral administration, the compounds or conjugates described herein may be combined with saline, PBS, or other suitable buffer, at an appropriate pH. A sustained release formulation can also be used for either or both therapeutic agents.

The compositions may be administered in oral dosage forms such as tablets, capsules (each of  
25 which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular forms, all utilizing dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compositions of the invention may be administered by intranasal route via topical use of suitable intranasal vehicles, or via a transdermal route, for example using  
30 conventional transdermal skin patches. A dosage protocol for administration using a transdermal delivery system may be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen of the invention will vary depending upon known factors such as the pharmacodynamic characteristics of the agents and their mode and route of administration; the species, age, sex, health, medical condition, and weight of the patient, the nature and extent of the symptoms, the kind of  
35 concurrent treatment, the frequency of treatment, the route of administration, the renal and hepatic function of the patient, and the desired effect. The effective amount of a drug required to prevent, counter, or arrest progression of a condition can be readily determined by an ordinarily skilled physician or veterinarian.

An amount of a therapeutic of the invention which will be effective in the treatment of a particular condition or disorder will depend on the nature of the condition or disorder, and can be determined by  
40 standard clinical techniques. The precise dose to be employed in the formulation will also depend on the

route of administration, and the seriousness of the condition or disorder, and should be decided according to the judgement of the practitioner and each patient's circumstances. Routine determinations of blood levels of insulin or C peptide, and of fasting levels of glucose or glucose challenges, are determined by one of ordinary skill in the art. Suitable dosage ranges for administration are generally about 0.01 micrograms to about 10,000 micrograms of each active compound per kilogram body weight per day, for example, about 0.01 micrograms to about 1 microgram/kg, about 0.1 micrograms/kg to about 10 micrograms/kg, about 1 microgram/kg to about 500 micrograms/kg, or about 10 micrograms/kg to about 10 mg/kg of body weight per day. Suitable dosage ranges for administration are thus generally about 0.01 micrograms/kg body weight/day to about 10 mg/kg body weight/day.

Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

A composition or treatment of the invention may comprise a unit dosage of at least one KGF agonist and a unit dosage of at least one gastrin compound. A "unit dosage" refers to a unitary i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active agents as such or a mixture with one or more solid or liquid pharmaceutical excipients, carriers, or vehicles.

In an aspect, a pharmaceutical composition is provided comprising a therapeutically effective suboptimal dosage of a KGF agonist and a gastrin compound that are more effective at decreasing or reducing glucose levels for a sustained period or increasing beta cell proliferation or differentiation following treatment compared with a dosage of either a gastrin compound or KGF agonist alone.

In another aspect, an improved pharmaceutical composition is provided comprising therapeutically effective suboptimal amounts of a KGF agonist and a gastrin compound in a form for chronic or acute therapy of a disease or condition, in particular diabetes.

In an embodiment, the composition comprises a KGF agonist and a gastrin compound in doses that are equal to or at least 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound required to provide beneficial effects, preferably sustained beneficial effects, to treat a disease or condition.

In an aspect the invention provides a pharmaceutical composition comprising between 0.5 to 6000, 100-1500, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms KGF agonist per single unit and 0.5 to 6000, 100-3000, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms gastrin compound per single unit.

In another aspect the invention provides a pharmaceutical composition comprising between 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 100 micrograms/kg/day KGF agonist and 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day gastrin compound.

A composition or formulation of the invention may be administered to a subject for 2 weeks to 12 months, 2 weeks to 6 months, 2-16 weeks, 2 weeks to 12 weeks, and/or 2-8 weeks, or periodically.

In an embodiment, the ratio of KGF agonist to gastrin compound in a composition of the invention is selected to augment the activity of the KGF agonist and/or gastrin compound and to provide beneficial effects, preferably sustained beneficial effects.

A KGF agonist and a gastrin compound may be in a ratio selected to augment the activity of one or both compounds to produce beneficial effects, preferably an additive or synergistic effect, or beneficial

effects, preferably sustained beneficial effects. In particular embodiments, the ratio of a KGF agonist to a gastrin compound may be from 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1. In other particular embodiments, the ratio of a gastrin compound to a KGF agonist may be from 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5.

- 5 In an embodiment, a KGF agonist may be used in combination with a gastrin compound at therapeutically effective weight ratios of between about 1:1 to 1:150, preferably 1:1 to 1:50. In another embodiment, a gastrin compound may be used in combination with a KGF agonist at therapeutically effective weight ratios of between about 1:1 to 1:150, preferably 1:1 to 1:50.

- The compositions of the present invention or fractions thereof typically comprise suitable  
10 pharmaceutical diluents, excipients, vehicles, or carriers selected based on the intended form of administration, and consistent with conventional pharmaceutical practices. The carriers, vehicles etc. may be adapted to provide an additive, synergistically effective or therapeutically effective amount of the active compounds.

- Suitable pharmaceutical diluents, excipients, vehicles, and carriers are described in the standard  
15 text, Remington's Pharmaceutical Sciences, Mack Publishing Company. By way of example for oral administration in the form of a capsule or tablet, the active components can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, methyl cellulose, magnesium stearate, glucose, calcium sulfate, dicalcium phosphate, mannitol, sorbitol, and the like. For oral administration in a liquid form, the drug components may be combined with any oral, non-toxic,  
20 pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Suitable binders (e.g. gelatin, starch, corn sweeteners, natural sugars including glucose; natural and synthetic gums, and waxes), lubricants (e.g. sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride), disintegrating agents (e.g. starch, methyl cellulose, agar, bentonite, and xanthan gum), flavoring agents, and coloring agents may also be combined in the compositions or components thereof.  
25 Compositions as described herein can further comprise wetting or emulsifying agents, or pH buffering agents.

- The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The compositions can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as  
30 pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Various delivery systems are known and can be used to administer a composition of the invention, e.g. encapsulation in liposomes, microparticles, microcapsules, and the like.

In an aspect of the invention a pharmaceutical composition has a pH from about 7 to 10.

- Formulations for parenteral administration of a composition of the invention may include aqueous  
35 solutions, syrups, aqueous or oil suspensions and emulsions with edible oil such as cottonseed oil, coconut oil or peanut oil. Dispersing or suspending agents that can be used for aqueous suspensions include synthetic or natural gums, such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose, and polyvinylpyrrolidone.

- Compositions for parenteral administration may include sterile aqueous or non-aqueous solvents,  
40 such as water, isotonic saline, isotonic glucose solution, buffer solution, or other solvents conveniently used

for parenteral administration of therapeutically active agents. A composition intended for parenteral administration may also include conventional additives such as stabilizers, buffers, or preservatives, e.g. antioxidants such as methylhydroxybenzoate or similar additives.

5 In an embodiment, a composition herein is formulated in accordance with routine procedures as a pharmaceutical composition adapted for subcutaneous or intravenous administration to human beings. Typically, compositions for subcutaneous or intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ameliorate pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry, lyophilized powder or water-free  
10 concentrate in a hermetically sealed container such as an ampoule or sachette, for example, indicating the quantity of active agent. Where the composition is to be administered by infusion, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

Compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids etc., and those formed with free carboxyl groups such as those  
15 derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

In an embodiment, a solid form pharmaceutical composition is provided (e.g. tablets, capsules, powdered, or pulverized form) comprising a crystalline or amorphous KGF agonist and a gastrin compound.

20 In another embodiment, a solid form pharmaceutical composition is provided (e.g. tablets, capsules, powdered, or pulverized form) comprising a crystalline or amorphous a crystalline or amorphous gastrin compound and a KGF agonist.

In another embodiment, the invention relates to a liquid drug formulation comprising pharmaceutically acceptable salts of a KGF agonist and a gastrin compound, and to lyophilized drug  
25 formulations that can be reconstituted to provide suspensions that are stable and suitable for parenteral administration.

In a particular embodiment, the invention relates to an aqueous composition comprising pharmaceutically acceptable salts of a KGF agonist and a gastrin compound, and a solvent system which effects solubilization. The invention also provides a drug comprising an aqueous formulation of  
30 pharmaceutically acceptable salts of a KGF agonist and a gastrin compound with at least one solubilizer.

A composition of the invention may be sterilized by, for example, filtration through a bacteria retaining filter, addition of sterilizing agents to the composition, irradiation of the composition, or heating the composition. Alternatively, the compounds, conjugates, and compositions of the present invention may be provided as sterile solid preparations e.g. lyophilized powder, which are readily dissolved in sterile solvent  
35 immediately prior to use.

In addition to the formulations described herein, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the fractions may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable  
40 oil), or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.



The compositions of the invention and components thereof may comprise soluble polymers as targetable drug carriers.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labelled for treatment of an indicated condition. For administration of a composition of the invention, such labelling would include amount, frequency, and method of administration.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates or as compositions, the invention also provides a kit comprising a KGF agonist and a gastrin compound, a pharmaceutical composition or conjugate in kit form. The invention also provides a pharmaceutical kit comprising one bottle with a KGF agonist and another bottle with a gastrin bottle in one box.

In embodiments of the invention, a pharmaceutical pack or kit is provided comprising one or more containers filled with one or more of the ingredients of a pharmaceutical composition of the invention. Associated with such container(s) can be various written materials such as instructions for use, or a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

According to another aspect of the invention, a kit is provided. The kit is a package which houses a container which contains a covalent conjugate of the invention and also houses instructions for administering the covalent conjugate to a subject.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

#### Examples

##### Example 1. Experimental schedule for preparation of Streptozotocin diabetic rats, and treatment with KGF/gastrin

Male Wistar rats will be injected with 30 mg/kg of streptozotocin intravenously (iv.) on three consecutive days. Glycemia will be controlled by insulin pellets implanted subcutaneously before treatment (one pellet per rat). Blood glucose measurements will be taken prior to implanting insulin pellets subcutaneously (sc) and non-fasting blood glucose levels will be monitored to determine the degree of glucose control. The KGF and gastrin combination (50 and 300 µg/kg, respectively) will be administered as a subcutaneous bolus injection 3 times daily for 4 weeks, commencing three weeks after injection of the STZ injection. An intraperitoneal glucose tolerance test (IPGTT) will be conducted 6 days after cessation of treatment. Further, C-peptide and pancreatic insulin can be assessed.

##### Example 2. Assay of non-fasting blood glucose

Using rats from Example 1, treating rats with a composition which is a combination of KGF/gastrin should improve the non fasting blood glucose level in diabetic rats, compared to those treated with vehicle alone. Further, following an intraperitoneal glucose challenge, blood glucose should return to a more normal level more quickly in the KGF and gastrin treated rats.

##### Example 3. Assay of glucose tolerance and insulin secretion

Using the streptozotocin rats from Example 1, it can be demonstrated that treatment with KGF/gastrin combination should improve insulin secretion and C peptide release. Improvement in glucose tolerance of the diabetic rats treated with KGF/gastrin should result from increased insulin secretion.

Example 4. KGF Gastrin treatment increases pancreatic  $\beta$  cell mass

5 Rats will be made diabetic with 65mg/kg streptozotocin (STZ) at a time point 10 days prior to treatment, the treatment comprising 250  $\mu$ g/kg of KGF each day and 250  $\mu$ g/kg each day of gastrin, administered by subcutaneous infusion for 2 weeks. An identically diabetic control group will receive vehicle by a subcutaneous infusion route similar to that of the KGF/gastrin group. One week after the end of the treatment period, the rats will be sacrificed and the pancreas will be collected for insulin determination and  $\beta$  cell mass determination.

10 The  $\beta$ -cell mass will be determined by point counting morphometrics according to the following protocol. Paraffin sections (5  $\mu$ m) are immunoperoxidase stained for insulin determination. Diaminobenzidine is used as the substrate for peroxidase and slides are counterstained only with hematoxylin. One section from each pancreas is examined by point counting. A rectangular 16x12 point grid is used, total 192 points. Each pancreas section is counted using the 10x magnification lens. The field is projected to a computer screen, final magnification 265x. At this magnification the diameter of Beta-cells is 3-5 mm. Total number of points counted per section is typically 11,000 – 20,000 depending on the size of the section. The  $\beta$ -cell content is calculated by dividing the number of points falling on insulin stained cells by the total number of points falling on pancreatic tissue. The  $\beta$ -cell content is expressed as a percent. The  $\beta$ -cell mass is calculated by multiplying  $\beta$ -cell content (%) with the weight of the pancreas, in mg. Pancreatic insulin content is determined by immunoassay of ethanol water extracts of pancreas.

STZ causes severe depletion of  $\beta$ -cell mass in comparison to those in normal (untreated) rats.

After 7 days of treatment with the KGF/Gastrin combination, there should be an increase in  $\beta$  cell mass. A correlation should also be obtained between  $\beta$ -cell mass and insulin content. Thus, increased pancreatic insulin content will result from a greater  $\beta$ -cell mass.

Example 5. The combination of gastrin with KGF enhances the potency of KGF

30 Rats will be made diabetic with streptozotocin administered intravenously (60 mg/kg), and 14 days later (rather than the 21 day interval of Examples, supra), rats will be distributed into 4 groups: 10  $\mu$ g/kg KGF administered via the intraperitoneal route (ip) twice daily, 10  $\mu$ g/kg Gastrin ip twice daily, combination of each of 10  $\mu$ g/kg KGF and 10  $\mu$ g/kg Gastrin ip twice daily, or Vehicle ip twice daily.

Before treatment, blood glucose levels are restored to nondiabetic levels by depot insulin replacement therapy. At the end of the 10 day treatment period, an IPGTT will be performed, and the results should show that only rats given the KGF /gastrin combination have improved glucose tolerance compared to rats administered only vehicle.

Example 6. Long term efficacy of KGF/Gastrin treatment

40 After a 10 day course of treatment with the KGF/Gastrin combination, treated Stz rats should show prolonged improvement of levels of fasting blood glucose, compared to untreated diabetic rats in the vehicle group (STZ rats having a similar level of diabetes before treatment with vehicle alone). Rats will be treated with 10  $\mu$ g/kg KGF and 10  $\mu$ g/kg gastrin by ip injection twice daily for 10 days. The rats will be followed

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for up to 4 months measuring both the fasting blood glucose and the glucose tolerance after an IPGTT. Treatment of rats with a KGF/Gastrin combination will cause a significant reduction in fasting blood glucose compared to the untreated diabetic STZ rats, so that the fasting blood glucose level more resembles that of the non-diabetic control rats. Even at a time such as four months after treatment, blood glucose in treated STZ rats should be significantly lower than that of untreated control rats.

Example 7. Treatment with KGF and gastrin prevents disease progression in NOD mice with recent-onset diabetes.

The non-obese diabetic (NOD) strain of mouse is an excellent model of autoimmune (type 1) diabetes, as the phenotype mirrors many features of disease pathogenesis in the human form of type 1 diabetes. NOD mice typically exhibit destructive autoimmune pancreatic insulinitis and  $\beta$ -cell destruction as early as four weeks of age. Diabetes onset usually occurs at age 10-15 weeks in these mice, with typical blood glucose levels observed to be between 7-10 mM (compared to a range of about 3.0-6.6 mM normal mice), and a pancreatic insulin level lower by more than about 95% than that in normal mice. As the disease progresses, NOD mice exhibit increasingly severe signs of chronic diabetes, with blood glucose levels reaching between about 25 to about 30 mM and pancreatic insulin level declining to become virtually non-existent. At that severe stage of the disease, greater than about 99% of the  $\beta$ -cells have been destroyed.

In this example, the effect of treatment by a combination of KGF + gastrin is examined in NOD mice with recent onset diabetes. Specifically, the objective is to determine whether administration of low doses of both KGF and gastrin prevents severe hyperglycemia and increases pancreatic insulin content in NOD mice with recent-onset diabetes. Gastrin is synthetic human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15, and KGF is recombinant human KGF.

Non-obese diabetic (NOD) female mice, ages 10-12 weeks, will be monitored for development of onset of diabetes (fasting blood glucose > 6.6 mmol/l), and within 36 hours after onset of symptoms, each of three groups of mice will be treated as follows: with vehicle; with 0.25  $\mu$ g/kg/day of KGF; with 1.5  $\mu$ g/kg/day of gastrin; or with 0.25  $\mu$ g/kg/day of KGF + 1.5  $\mu$ g/kg/day of gastrin, each treatment administered via the i.p. route twice daily. A range of from about 0.1  $\mu$ g/kg/day to about 100  $\mu$ g/kg/day of KGF and a range of about 1  $\mu$ g/kg/day to about 60  $\mu$ g/kg/day of gastrin can be used. Therapy is administered for 14 - 28 days. Upon cessation of therapy, animals are maintained untreated for an additional 10-14 days, and are sacrificed so as to determine whether prevention of severe hyperglycemia persists after termination of therapeutic treatment. Mice receive neither insulin-replacement treatment nor immunosuppression. The following parameters are assessed: survival rates, status of liver function, pancreatic insulin levels, and fasting blood glucose levels.

In animals in the vehicle-treated control group, it is known that fasting blood glucose (FBG) values increase progressively during the time course of the treatment, and that a majority of the vehicle-treated mice will eventually die from severe hyperglycemia and ketoacidosis. In contrast, all mice in the group treated with KGF and gastrin should survive, and their FBG values should be significantly less than in the vehicle-treated mice. Improved control of blood glucose levels in mice treated with KGF and gastrin may be associated with a significantly increased content of insulin in the pancreas of these mice. Treatment with KGF and gastrin should be more effective than treatment with KGF alone for the parameters measured.

In summary, the Examples can show that treatment with a short course of low doses of KGF and gastrin treatment in mice with recent onset of diabetes prevents disease progression and improves pancreatic insulin content, and these effects are sustained for a prolonged period of time after termination of therapy. Treatment with the combination of KGF and gastrin is expected to yield a reduction in fasting blood glucose levels and an increase in pancreatic insulin content that is greater than the changes in these parameters in mice treated with KGF alone. A lower concentration of KGF can be used in combination with gastrin than alone, which can ameliorate the known side effects of KGF at higher concentrations.

Treatment with KGF and gastrin prevents and/or compensates for the autoimmune destruction of pancreatic islet  $\beta$ -cells, thereby maintaining the  $\beta$ -cell mass and pancreatic insulin content at a level sufficient to prevent severe hyperglycemia, ketoacidosis, and death. Further, administration of both KGF and gastrin prevents progression in mice with recent-onset diabetes, without concurrent insulin replacement and immunosuppression.

Example 8 Effects of Gastrin and Keratinocyte Growth Factor (KGF) in treating Acutely-Diabetic Non-Obese Diabetic (NOD) Mice

This study aimed to inhibit the progression of diabetes in diabetic NOD mice *in vivo* through systemic treatment with gastrin and KGF. Female NOD mice ages 12-14 weeks were treated for 18 days with vehicle (PBS), or KGF 0.25 ug/kg/day + gastrin 1.5 ug/kg/day, by injection intraperitoneally within 2 days after diabetes onset and with fasting blood glucose (FBG) levels of 9-15 mM (normal FBG <6.6 mM). There were a total of 4 mice per group.

After 18 days of treatments, FBG was  $18.3 \pm 4.2$  mM (mean  $\pm$  SE) in vehicle-treated mice. In contrast, the FBG of the treated group was significantly lower than that of vehicle-treated mice. FBG was  $10.2 \pm 1.0$  mM (mean  $\pm$  SE) in mice treated with the combination of KGF and gastrin. (Figure 1)

Thus, a short course of KGF and gastrin treatment of diabetic NOD mice with recent onset diabetes reduces hyperglycemia and inhibits progression of diabetes.

The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

Table 1

5 Keratinocyte Growth Factor Analogs and Derivatives

Patent	Title	Analog/Derivative
EP0941110B1  Inventors: LACEY, David, L.; ULICH, Thomas, R.; DANILENKO, Dimitry, M.; FARRELL, Catherine, L.  Assignee: Amgen  Also: WO9816243A1 US20030144202A1	Uses of keratinocyte growth factor 2	A protein defined by amino acids Cys37 to Ser208 of mature KGF-2 and variant proteins thereof that retain biological activity  Mutation of sequence of KGF2 in regions of low homology to other FGF family members (table lists preferred substitutions)  Adding to the N or C terminal or internal additions, e.g. fuse a signal sequence to the N terminal or a heavy or light chain IgG to the C terminal
EP0785950B1  Assignee: Amgen  Also: EP0785948B1 WO9611951A2 US5858977	Keratinocyte growth factor analogs	KGF analogues: deletion and/or substitution of one or more of amino acid residues 41-154; optionally further having a deletion of the first 15 to 24 amino acids of the N-terminal native KGF, having residues corresponding to Cys (1) and Cys (15) replaced or deleted, having an N-terminal methionine or having a signal sequence, with the proviso that the amino acid sequence 123-131 of the above sequence is not substituted by any one of the amino acid sequences selected from the group of DLYQG and AKYEG
EP1012186B1  Assignee; Amgen  Also: WO9824813A2	Use of a kgf protein product(s) and a glp-2 protein product(s) for the preparation of a medicament	KGF protein product(s) is selected from the group consisting of C(1,15)S, DELN15, DELN16, DELN17, DELN18, DELN19, DELN20, DELN21, DELN22, DELN23, DELN24, DELN3/C(15)S, DELN3/C(15)-, DELN8C(15)S, DELN8/C(15)-, C(1,15)S/R(144)E, C(1,15)S/R(144)Q and DELN23/R(144)Q.

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<p>US5858977</p> <p>Inventor: Aukerman, Sharon Lea Pierce, Glenn Francis; Rancho</p> <p>Assignee: Amgen Inc</p>	<p>Method of treating diabetes mellitus using KGF</p>	<p>Amino acid residues 32-194 of native KGF) or an analog thereof which comprises a difference from the sequence of native KGF by having an amino acid deletion of or having a residue other than cysteine substituted for Cys32 and Cys46</p> <p>The patent lists a number of other deletion mutants</p>
<p>WO9622369A1</p> <p>Inventors: ARAKAWA, Tsutomu; FOX, Gary, Michael;</p> <p>Assignee: Amgen</p>	<p>Analog of acidic fibroblast growth factor having enhanced stability and biological activity</p>	<p>Substitute at least one amino acid of KGF (in the region of amino acids 115-119 of Figures 9 &amp; 10) having higher loop-forming potential for an amino acid residue of lower loop-forming potential in or about the loopforming sequence Asn-His-Tyr-Asn-Thr-Tyr of the naturally occurring protein.</p>
<p>WO9611949A2</p> <p>Inventors: MORRIS, Charles, F.; KENNEY, William, C.; CHEN, Bao-Lu; HSU, Eric, W</p> <p>Assignee: Amgen and inventors</p> <p>Also: WO9611950A1 US5858977</p>	<p>Analog of keratinocyte growth factor</p>	<p>KGF having up to the first 24 N-terminal amino acids modified wherein cysteine residues corresponding to amino acid positions 1 and 15 of the KGF amino acid positions 32 and 46 of SEQ ID No:2, (Cys&lt;1&gt; and Cys&lt;15&gt;, respectively) are deleted or substituted with another amino acid</p> <p>Also listed are: C(1,15)S, NA15, NA16, NA17, NA18, NA19, NA20, NA21, NA22, NA23 and NA24, AN3/C(15)S, AN3/C(15)-, AN8/C(15)S, AN8/C(15)-, C(1f15)S/R(144)EI C(1,15)S/R(144)Q and AN23/R(144)Q (included in patent EP1012186B1 above)</p>

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<p>EP0935652B1</p> <p>Inventors: NARHI, Linda, Owens; OSSLUND, Timothy, D.</p> <p>Assignee: Amgen</p> <p>Also: WO9816642A1 US20040043924A1</p>	<p>Keratinocyte growth factor-2 products</p>	<p>KGF-2 defined by amino acids Cys37 to Ser208 of SEQ ID NO:2 and variant proteins thereof, including a protein in which one or more amino acid residues have been deleted from, inserted into, and/or substituted for residues within the amino acid sequence of SEQ ID NO:2 and which retains biological activity</p> <p>In particular NH<sub>2</sub>-Ser-Tyr-[Asn71-Ser208]-COOH or NH<sub>2</sub>-Ala-Gly-Arg-His-Val-Arg-Ser-Tyr-[Asn71-Ser208]-COOH</p> <p>WO9816642A1 includes the above with the following included in the claims section: R1-[Asn71-Pro203]-COOH proteins where a long list of possible amino acids representing R1 includes Tyr Ser-Tyr Arg-Ser-Tyr Val-Arg-Ser-Tyr (SEQ ID NO:9) and many others and R2 carboxy terminal amino acids</p>
<p>US6677301</p> <p>Inventors: Gospodarowicz, Denis J. Masiarz, Frank R.;</p> <p>Assignee: Chiron Corporation,</p> <p>Also: US5863767 US5843883 US6074848 US5773586 US5677278 WO9501434A1</p>	<p>Truncated keratinocyte growth factor (KGF) having increased biological activity</p> <p>Derwent Title: New keratinocyte growth factor with N-terminal deletion can form conjugates with toxins, - also DNA vectors and transformed cells, useful for stimulating wound healing and treating epidermal hyperproliferat ion</p>	<p>KGF163 with the N terminal 23 amino acids deleted</p> <p>Deletion of KGF reported to show increased biological activity and less cytotoxicity to epithelial cells</p> <p>Also includes amino acid insertions, deletions and substitutions and analogues that retain biological activity and at least a two-fold increase in mitogenic activity over kgf163</p>

<p>US5677278</p> <p>Inventor: Gospodarowicz, Denis J.; Masiarz, Frank R.</p> <p>Assignee: Chiron Corporation</p> <p>Also: US6677301 US6074848 US5863767 US5843883 US5773586</p>	<p>Truncated keratinocyte growth factor (KGF) having increased biological activity</p> <p>Derwent Title: New keratinocyte growth factor with N-terminal deletion can form conjugates with toxins, - also DNA vectors and transformed cells, useful for stimulating wound healing and treating epidermal hyperproliferat ion</p>	<p>As in WO03095637A1 (above)</p>
<p>US20030109439A1</p> <p>Inventors: Gospodarowicz, Denis J.; Kavanaugh, W. Michael; Crawford, Kenneth</p> <p>No Assignee but the Attorney/Agent/ Firm is Chiron</p> <p>Also: WO03016505A2</p> <p>Assignee: Chiron</p>	<p>KGF polypeptide compositions</p> <p>Derwent Title: Use of the keratinocyte growth factor polypeptide for the manufacture of a medicament for stimulating epithelial cell proliferation</p>	<p>KGF polypeptides include KGF des1-15 i.e. . amino acids 16-163, KGFdes1-18 through to KGFdes1-22, KGFdes1-24 and KGFdes1-25, also biologically active analogues of these, where these biologically active analogues consists of the same number of amino acids respectively, and have at least 70% sequence homology and exhibits an increase in bioactivity relative to mature, full- length, KGF (KGF163)</p>
<p>WO9713857A1.</p> <p>Inventors: Williams, Lewis</p> <p>Assignee: Chiron</p>	<p>Combination pdgf, kgf, igf and igfbp for wound healing</p>	<p>KGF refers to any one of a mature polypeptide and biologically active fragments, analogs, and derivatives thereof as described in WO 90/08771 and WO 95/01434.</p>



<p>US6693077</p> <p>Inventors: Stephen Ruben et al</p> <p>Assignee: Human Genome Science</p> <p>Also: EP0950100B1 WO0102433A1 US20030129687A1 US20030077695A1 US6077692</p>	<p>Keratinocyte growth factor 2.</p> <p>Keratinocyte growth factor-2 deletion mutants - useful to promoter or accelerate wound healing</p>	<p>Includes: recombinant, natural and synthetic forms of KGF-2, fragment, derivative or analogues of KGF2 including amino acid substitutions , allelic variations, deletions in sequence, fusion to molecules that increase half- life, amino acid additions to assist in purification etc. mimetic peptides and KGF 2 variants that show biological activity similar to KGF2, recombination with heterologous molecules</p> <p>Multimers of any of the above or the addition of for example, a leucine zipper peptide to induce multimer formation</p> <p>Antigenic epitope bearing peptides will be used to raise antibodies</p>
<p>US6653284</p> <p>Inventors: Gentz R et al</p> <p>Assignee: Human Genome Science</p> <p>Also: US6599879 US6238888 WO9941282A1 WO9932135A1</p>	<p>Keratinocyte growth factor-2 formulations</p>	<p>This patent includes KGF2 and deletion mutants of KGF2 including N teminal deletions (e.g. Ala(63)-Ser(208) and Ser(69)-Ser(208)), C terminal deletions and both N&amp;C terminal deletions, conservative amino acid substitutions</p> <p>Lists of substitutions and deletions are given in the patent</p>
<p>EP0815115B1</p> <p>Assignee: Human Genome Science</p> <p>Also: US20030129687A1 US20030077695A1 US6693077 US6077692</p>	<p>Keratinocyte growth factor-2</p>	<p>KGF-2 sequences are amino acids 36 -172 and 1-172 (amino acids 1-36 are the signal sequence) or the full length polypeptide</p>

<p>EP1247862A2</p> <p>Assignee: Human Genome Science</p> <p>Also: EP1247530A2 WO02077155A2 WO9806844A1 US20030129687A1 US20030077695A1 US6693077 US6077692</p>	<p>Keratinocyte growth factor-2 (KGF-2 or fibroblast growth factor-12, FGF-12)</p> <p>Derwent Title Keratinocyte growth factor-2 deletion mutants - useful to promoter or accelerate wound healing</p>	<p>KGF-2 Nterminal deletion mutants including deleting at least 38 but less than 137 amino acids; KGF-2 C terminal deletion mutants deleting at least one and up to 55 amino acids where the N-terminal amino acid residue of said KGF-2 C-terminal deletion mutant is amino acid residue 1 (Met), 36 (Thr), or 37 (Cys); N and C terminal mutants; there is a long list given in the claims of other mutants</p>
<p>US6077692</p> <p>Inventor: Ruben, S et al</p> <p>Assignee: Human Genome Science</p> <p>Also: US20030186904A1 US20030129687A1 US20030077695A1 US6693077</p>	<p>Keratinocyte growth factor-2</p> <p>Derwent Title: Novel keratinocyte growth factor useful for promoting and accelerating wound healing, comprising at least 10 contiguous amino acids from a specific amino acid sequence</p>	<p>Some preferred embodiments include the N-terminal deletions Ala (63)--Ser (208) (KGF-2.DELTA.28) (SEQ ID NO:68) and Ser (69)--Ser (208) (KGF-2.DELTA.33) (SEQ ID NO:96). Other preferred N-terminal and C-terminal deletion mutants include: Ala (39)--Ser (208) (SEQ ID NO:116); Pro (47)--Ser (208) of FIG. 1 (SEQ ID NO:2); Val (77)--Ser (208) (SEQ ID NO:70); Glu (93)--Ser (208) (SEQ ID NO:72); Glu (104)--Ser (208) (SEQ ID NO:74); Val (123)--Ser (208) (SEQ ID NO:76); and Gly (138)--Ser (208) (SEQ ID NO:78). Other preferred C-terminal deletion mutants include: Met (1), Thr (36), or Cys (37)--Lys (153)</p> <p>Included are deletion mutants of both N and C terminals as well as substituted amino acids</p>
<p>WO0170255A2</p> <p>Assignee: Pfizer and OSI Pharmaceuticals</p>	<p>Combined treatment with keratinocyte growth factor and epidermal growth factor inhibitor</p>	<p>KGF-1 or KGF-2 or analogs having at least partial human activity</p>

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<p>WO0149309A2</p> <p>Inventors: DAVIES, Michael, John; HUGGINS, Jonathan, Paul; MCINTOSH, Fraser, Stuart; OCCLESTON, Nicholas, Laurence</p> <p>Assignee: Pfizer</p>	<p>Composition for the treatment of damaged tissue</p>	<p>KGF or KGF-2 or active variants, homologues, derivatives or fragments thereof</p>
<p>US6709842</p> <p>Inventor: Rubin, Jeffrey S.; Finch, Paul W.; Aaronson, Stuart A</p> <p>Assignee: USA Dept of Health and Human Services</p> <p>Also: US6420531 US5731170 WO9008771A1 US5741642 US5707805 US5665870 US5654405</p>	<p>DNA encoding a growth factor specific for epithelial cells</p>	<p>Express KGF and KGF-like polypeptides, amino acids 32 to 194 or segment thereof given in Figure 7, including chimeras</p> <p>chimera e.g. 40 amino acids from the N - terminus of the secreted form of KGF (beginning with the amino terminal cys residue of the mature KGF form, numbered 3 in FIG. 7, and ending at KGF residue 78, arg) is linked to about 140 amino acids of the C-terminal core of <math>\alpha</math>FGF (beginning at residue 39, arg, and continuing to the C- terminal end of the <math>\alpha</math>FGF coding sequence. US5731170 includes KGF-like peptides with amino acids 65-156 and 162-189 of Figure 7 and a KGF that is truncated between amino acids 32-78 of the sequence of FIG. II-1B; chimeras are also given</p>

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<p>EP0555205B1</p> <p>Inventors/Assignees: Rubin, Jeffrey S.; Finch, Paul W.; Aaronson, Stuart A</p> <p>Also: WO9008771A1 US6709842 US6420531 US5741642 US5731170 US5707805 US5665870 US5654405</p>	<p>DNA encoding a growth factor specific for epithelial cells</p>	<p>Sequences and potential variants of KGF in the claims including full length kgf, glycosylated or unglycosylated, an N terminal deletion of 31 amino acids, addition/deletion or substitution of one or more amino acids in the sequence or segments of the sequence</p> <p>Other variations given</p>
<p>WO02094872A1</p> <p>Assignee: Human DNA Technology Inc.</p> <p>Also: US20030036174A1</p>	<p>Therapeutic uses of keratinocyte growth factor-</p>	<p>KGF-2 amino acids 40-208</p>
<p>WO02094871A1</p> <p>Assignee: Human DNA Technology Inc.</p> <p>Also: US20030036174A1</p>	<p>Novel keratinocyte growth factor-2 analogue in hair follicle</p>	<p>KGF-2 analog (KGF-2A): substituted Glu for Lys at the 87 th codon of the amino acid sequence of KGF, amino acids 40-208 or 2-208 or 1-208; M at the N terminus of the sequence</p>
<p>US20040063634A1</p> <p>Inventors: Carr, Francis J.; Graham, Carter; Jones, Tim; Williams, Stephen</p>	<p>Modified keratinocyte growth factor (kgf) with reduced immunogenicity</p>	<p>Immune characteristic is modified by means of reduced or removed numbers of potential T-cell epitopes</p> <p>Substitute one or more amino acids (table gives list of potential substitutions)</p>

<p>WO03095637A1</p> <p>Inventors: Ni, Yawei et al</p> <p>Assignee: Carrington Laboratories Inc.</p> <p>Also: US20030147876A1</p>	<p>Combination of a growth factor and a protease enzyme</p> <p>Derwent Title: A new composition containing comprising growth factor related to epithelial cell function and an extracellular matrix degrading enzyme is useful to treat injury of skin, oral cavity, digestive track, mucosal surface, eye or lung</p>	<p>The growth factor related to kgf is most likely A23KGF, which is KGF with the first 23 amino acids deleted, which exhibits at least a 2-fold increase in mitogenic activity as compared to a mature full-length keratinocyte growth factor (disclosed in US5677278 and incorporated into this patent - the summary follows)</p>
<p>WO0072872A1</p> <p>Assignee: KHAN, Fazal</p>	<p>Keratinocyte growth factor-2 formulations</p>	<p>Preferred embodiments include the N-terminal deletions Ala (63) -- Ser (208) (KGF-2A28) and Ser (69) -- Ser (208) (KGF-2A33).</p> <p>A list of N, C or N&amp;C terminal deletion mutants is provided</p>

WHAT IS CLAIMED IS:

- 5 1. A pharmaceutical composition comprising a KGF agonist and a gastrin compound that provides beneficial effects relative to each compound alone, and optionally a pharmaceutically acceptable carrier, excipient, or vehicle.
2. A pharmaceutical composition as claimed in claim 1 that provides sustained beneficial effects.
- 10 3. A pharmaceutical composition as claimed in claim 2 in a form that provides normal blood glucose levels in a subject that persist for a prolonged period of time after administration.
4. A pharmaceutical composition as claimed in any preceding claim comprising therapeutically effective amounts of a KGF agonist and a gastrin compound in a form for chronic or acute therapy of a subject in need thereof.
- 15 5. A pharmaceutical composition as claimed in claim 4 wherein the therapeutically effective amounts are suboptimal relative to the amount of each compound administered alone for treatment of diabetes.
6. A pharmaceutical composition as claimed in any preceding claim wherein the ratio of KGF agonist to gastrin compound is selected to augment the activity of the KGF agonist or gastrin compound.
- 20 7. A pharmaceutical composition as claimed in claim 6 wherein the ratio of a KGF agonist to a gastrin compound is from about 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1.
- 25 8. A pharmaceutical composition as claimed in claim 6 wherein the ratio of a gastrin compound to a KGF agonist is from about 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5.
9. A pharmaceutical composition as claimed in any preceding claim wherein the KGF agonist is used in combination with the gastrin compound at therapeutically effective weight ratios of between about 1:1.5 to 1:150, preferably 1:2 to 1:50.
- 30 10. A pharmaceutical composition as claimed in any preceding claim wherein the KGF agonist and the gastrin compound are present in doses that are at least about 1.1 to 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound alone required to treat a disease or condition.
- 35 11. A pharmaceutical composition as claimed in claim 1 comprising an additive amount or synergistically effective amount of the KGF agonist and the gastrin compound in a pharmaceutically acceptable excipient, carrier, or vehicle.
- 40 12. A pharmaceutical composition as claimed in claim 1 comprising between 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day KGF agonist and 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day gastrin compound.

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13. A pharmaceutical composition as claimed in claim 2 wherein the beneficial effects are one or more of the following: reduced or absent islet inflammation, decreased disease progression, increased survival, or decreased symptoms of a disease or condition.
  14. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effects are sustained beneficial effects that persist for a prolonged period of time after termination of treatment.
  15. A pharmaceutical composition as claimed in claim 13 wherein the beneficial effects are sustained for at least about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.
  16. A pharmaceutical composition as claimed in claim 13 wherein the sustained beneficial effects may manifest as increased C-peptide production, increased pancreatic insulin production, and about normal or low blood glucose levels for a prolonged period following treatment.
  17. A pharmaceutical composition as claimed in any preceding claim wherein the sustained beneficial effects are statistically significant in terms of statistical analysis of an effect of a KGF agonist and a gastrin compound compared with the effects of each of the compounds.
  18. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels.
  19. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels.
  20. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is a decrease in blood glucose levels for a period of at least about about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.
  21. A pharmaceutical composition as claimed in any preceding claim wherein the KGF agonist is a KGF, KGF-2, fragments, analogs, and derivatives thereof, and active metabolites and prodrugs of KGF.
  22. A pharmaceutical composition as claimed in any preceding claim wherein the KGF agonist is an analog or derivative of KGF listed in Table 1.
  23. A method for preventing and/or treating a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one KGF agonist and a gastrin compound to produce a sustained beneficial effect.
  24. A method of treatment comprising administering to a subject a therapeutically effective amount of at least one KGF agonist in combination with administration of at least one gastrin compound which upon administration to a subject with symptoms of diabetes provides sustained beneficial effects.

25. A method as claimed in claim 24 wherein administration with of at least one KGF agonist in combination with administration of at least one gastrin compound provides sustained beneficial effects of at least one symptom of diabetes.
- 5 26. A method as claimed in claim 24 wherein therapeutically effective amounts of the KGF agonist and the gastrin compound are combined prior to administration to the subject.
27. A method as claimed in claim 24 wherein therapeutically effective amounts of the KGF agonist and the gastrin compound are administered to the subject sequentially.
- 10 28. A method as claimed in any preceding claim wherein therapeutically effective amounts of a KGF agonist and a gastrin compound are administered at a physiologically acceptable pH.
29. A conjugate comprising a KGF agonist linked to a gastrin compound to provide beneficial effects, in particular sustained beneficial effects.
- 15 30. A method of preparing a stable pharmaceutical composition of a KGF agonist comprising mixing a KGF agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the KGF agonist and adapted to provide beneficial effects preferably sustained beneficial effects.
- 20 31. A method of treating a condition or disease comprising administering a therapeutically effective amount of a KGF agonist and a gastrin compound, or a composition or conjugate of any preceding claim to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.
- 25 32. A method of treating a condition or disease comprising administering a KGF agonist and a gastrin compound, or a composition or conjugate of any preceding claim with a plurality of cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.
- 30 33. A method for treating a subject with a condition or disease comprising contacting *ex vivo* a plurality of cells with a KGF agonist and a gastrin compound, or a composition or conjugate of any preceding claim, optionally culturing the cells, and administering the cells to the subject in need thereof.
- 35 34. A method of any preceding claim wherein the condition or disease is dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, Alzheimer's disease and other central and peripheral neurodegenerative conditions chronic heart failure, fluid retentive states, metabolic syndrome and related diseases, and disorders and obesity.
- 40 35. A method for inducing islet neogenesis in a subject comprising contacting islet precursor cells with a KGF agonist and a gastrin compound, or a composition, or



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conjugate of any preceding claim in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

36. A method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective amount of a KGF agonist and a gastrin compound or a composition or conjugate of any preceding claim.
37. Use of a composition comprising a combination of at least one KGF agonist and at least one gastrin compound for the preparation of a medicament for the treatment of a condition or disease.
38. A use of claim 37 wherein the condition or disease is dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, Alzheimer's disease and other central and peripheral neurodegenerative conditions chronic heart failure, fluid retentive states, metabolic syndrome and related diseases, and disorders and obesity.
39. A kit form of a composition or conjugate as claimed in any preceding claim.
40. A method for preventing and/or treating diabetes, the method comprising administering to a mammal in need thereof a composition comprising a combination of a KGF receptor ligand and a gastrin /CCK receptor ligand, in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal, thereby preventing and/or treating the diabetes.
41. A method for preventing and/or treating diabetes, the method comprising administering to a mammal in need thereof a composition comprising a combination of a KGF receptor ligand and a gastrin /CCK receptor ligand, in an amount sufficient to increase proliferation of islet precursor cells in pancreatic tissue, thereby preventing and/or treating the diabetes.
42. A method for preventing and/or treating diabetes, the method comprising: contacting *ex vivo* a plurality of cells with a composition comprising a KGF receptor ligand and a gastrin/CCK receptor ligand in an amount sufficient to increase proliferation of islet precursor cells and the amount of insulin secreting islet cells; and administering the contacted plurality of cells to a mammal in need thereof, thereby preventing and/or treating the diabetes.
43. A method of claim 42, wherein the amount of KGF in the composition is substantially lower than the minimum effective dose of KGF required to reduce blood glucose in the diabetic mammal in the absence of a gastrin/CCK receptor ligand.
44. A method for preventing and/or treating diabetes, the method comprising administering to a mammal in need thereof a composition comprising a combination of a KGF receptor ligand and a gastrin /CCK receptor ligand, in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal; and

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determining the amount of islet neogenesis, thereby preventing and/or treating the diabetes.

- 5 45. A method of claim 44, wherein determining the amount of islet neogenesis is measuring a parameter selected from the group of: blood glucose, serum glucose, blood glycosylated hemoglobin, pancreatic  $\beta$  cell mass, serum insulin, pancreatic insulin content, and morphometrically determined  $\beta$  cell mass.
- 10 46. A method of claim 45, wherein administering the composition reduces blood glucose compared to blood glucose assayed prior to administering the composition.
47. A method of claim 45, wherein administering the composition reduces blood glucose by 50% compared to blood glucose assayed prior to administering the composition.
- 15 48. A method of claim 45, wherein glycosylated hemoglobin concentration is reduced compared to glycosylated hemoglobin concentration in the control mammal not administered the composition.
49. A method of claim 45, wherein serum C peptide insulin concentration is increased compared to serum insulin concentration in the mammal assayed prior to administering the composition.
- 20 50. A method of claim 45, wherein pancreatic insulin concentration is increased compared to pancreatic insulin concentration in the mammal assayed prior to administering the composition.
51. A method of claim 45 wherein the cells are pancreatic ductal cells.
52. A method of claim 45, wherein the KGF receptor ligand and the gastrin/CCK receptor ligand are provided in an amount sufficient to induce differentiation of the pancreatic islet precursor cells into glucose responsive insulin secreting islet cells.
- 25 53. A method of claim 45 wherein the KGF receptor ligand and the gastrin/CCK receptor ligand are provided in an amount sufficient to induce differentiation of the pancreatic islet precursor cells into glucose responsive insulin secreting islet cells.
54. A method of claim 45, wherein the composition is provided in an amount sufficient to effect differentiation of pancreatic islet precursor cells in pancreatic tissue into mature insulin secreting islet cells.
- 30 55. A method of claim 45, wherein the composition is provided in an amount sufficient to increase proliferation of pancreatic islet precursor cells.
56. A method for inducing pancreatic islet neogenesis in a mammal, the method comprising administering to the mammal a composition comprising a combination of a KGF receptor ligand and a gastrin /CCK receptor ligand, in an amount sufficient to increase proliferation of islet precursor cells in pancreatic tissue, thereby inducing pancreatic islet neogenesis.
- 35 57. A method of claim 56, wherein the plurality of cells are multicellular.
58. A method of claim 56 wherein the plurality of cells are delivered systemically to the mammal.

59. A method for inducing pancreatic islet neogenesis in a mammal, the method comprising administering a composition comprising a combination of a KGF receptor ligand and a gastrin /CCK receptor ligand, in an amount sufficient to increase the number of pancreatic insulin secreting  $\beta$  cells in the mammal.
- 5 60. A method for inducing islet neogenesis therapy in a cell of an animal, comprising contacting the cell with a nucleic acid sequence encoding a gastrin/CCK receptor ligand operably linked to an insulin promoter receptor ligand and a nucleic acid sequence encoding a KGF receptor ligand operably linked to a metallothionein promoter.
- 10 61. A method of claim 60, wherein the cell is a germ cell.
62. A method of claim 60, wherein the cell is an autologous cell cultured *ex vivo*.
63. A nucleic acid construct comprising a nucleic acid sequence encoding a mammalian KGF receptor ligand operably linked to a heterologous promoter and a nucleic acid sequence encoding a mammalian gastrin/CCK receptor ligand operably linked to a heterologous promoter.
- 15 64. A composition comprising a gastrin/CCK receptor ligand and a KGF receptor ligand.
65. A composition of claim 64, in a dosage effective for inducing proliferation of islet precursor cells into an increased amount of mature insulin secreting cells.
66. A composition of claim 64 in a dosage effective for inducing differentiation of an islet precursor cell into a mature insulin secreting cell.
- 20 67. A transgenic animal whose germ cells comprise a nucleic acid sequence encoding a mammalian KGF receptor ligand operably linked to a heterologous promoter and a nucleic acid sequence encoding a mammalian gastrin/CCK receptor ligand operably linked to a heterologous promoter.
- 25 68. A kit for preventing and/or treating diabetes, containing a composition comprising a gastrin/CCK receptor ligand and a KGF receptor ligand, a container, and instructions for use.
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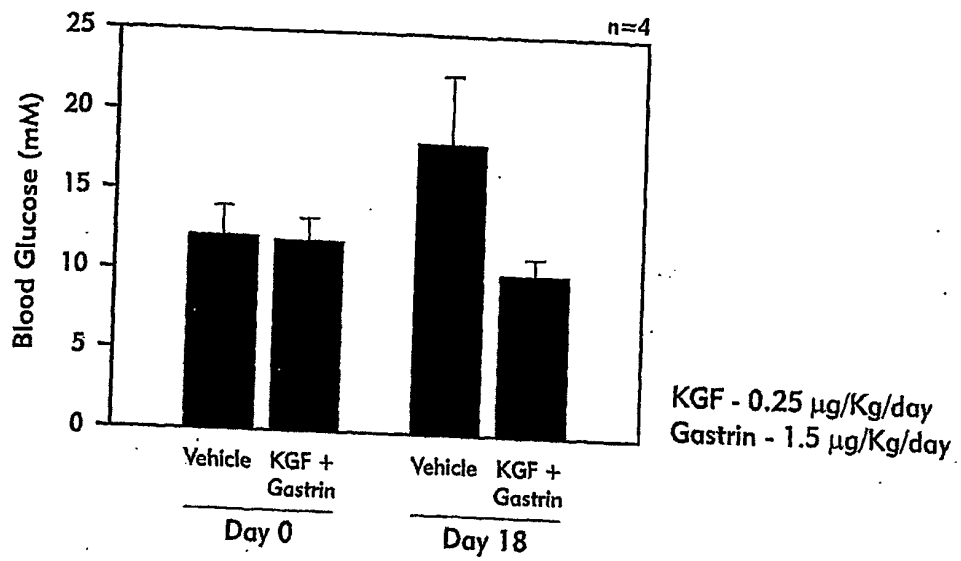


Figure 1

Sequence Listing

SEQ ID NO. 1

5 KGF (FGF-7)

Genbank Accession No. P21781 Homo sapiens

1 mhkwiltwil ptllyrscfh iiclvgtisl acndmtpeqm atnvcnsspe rhtrsydyme  
 10 61 ggdirvrrlf crtqwylrid krgkvkgtqe mknynimeir rtvavgivai kgvesefyla  
 121 mnkegklyak kecnedcnfk elilenhynt yasakwthng gemfvalnqk gipvrgekktk  
 181 keqktahflp mait

SEQ ID NO. 2

15 KGF (FGF-7)

Genbank Accession No. NP\_032034 and I48610 Mus musculus

20 1 mrkwiltil ptllyrscfh lvelvgtisl acndmspeqt atsvncsspe rhtrsydyme  
 61 ggdirvrrlf crtqwylrid krgkvkgtqe mknsynimeir rtvavgivai kgveseyyla  
 121 mnkegklyak kecnedcnfk elilenhynt yasakwthng gemfvalnqk gipvrgekktk  
 181 keqktahflp mait

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SEQ ID NO. 3

KGF-2 (FGF-10)

30 Genbank Accession No. BAA22331 Homo sapiens

1 mwkwilthca safphlpgcc cccflflfv svpvtcql gqdmvspeat nsssssfss  
 61 ssagrhvrs nhlggdvrrw rlfstkyf lkiekngkvs tkkencpysi leitsveig  
 121 vavkainsn ylamnkkgk lygskfndc kikerieeng yntyasfnwq hngrqmyval  
 35 181 ngkgaprrgq ktrrkntsa hlpmvhs

SEQ ID NO. 4

40 KGF-2 (FGF-10)

Genbank Accession No. AAH48229 Mus musculus

1 mwkwilthca safphlpgcc cccflflfv svpvtcql gqdmvsqeatn csssssfss  
 45 61 pssagrhvrs ynhlggdvrrw rlfstkyf lkiekngkvs gtnedcpys vleitsveig  
 121 vvavkainsn ylamnkkgk lygskfndc kikerieeng yntyasfnw qhngqmyva  
 181 lngkgaprrg qtrrkntsa hlpmtiq

50 SEQ ID NO. 5

N-terminal Glp-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-  
 Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

55 SEQ ID NO. 6

N-terminal Glp-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-  
 Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe

SEQ ID NO. 7

N-terminal Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe .

5 SEQ ID NO. 8

N-terminal Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe

SEQ ID NO. 9

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mqrivvyli falalaafse aswkprsqqp daplggtanr dlelpwleqq gpashhrrql  
gpqgpphlva dpskkqgpwl eeeeeaygwm dfgrrsaede n

SEQ ID NO. 10

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MCNDMTPEQMATNVNCSSPERHTRSVDYMEGGDIRVRRFCRTQWYLRIKRGKVKGTQEMKNN  
YNIMEIRTVAVGIVAIGVSEFYLAMNKEGKLYAKKECNEDCNFKELILENHYNTYASAKWTHNG  
GBMFVALNQKGIPVRGKKTKEQKTAHFLPMAIT

20 SEQ ID NO. 11

MLGQDMVSPE ATNSSSSSFS SPSSAGRHVR SYNHLQGDVR WRKLFSFTKY  
FLKIEKNGKV SGTCKENCYP SILEITSVEI GVVAVKAINS NYYLAMNKKG  
KLYGSKEFNN DCKLKERIEE NGYNTYASFN WQHNGRQMYV ALNGKGAPRR  
25 GQKTRRKNTS AHFLPMVVHS

## INTERNATIONAL SEARCH REPORT

International Application No.

T/CA2004/000648

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/595 C07K14/50 A61K38/33 A61K38/18 C12N15/09  
 A01K67/027 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K C12N A01K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, PASCAL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 858 977 A (AUKERMAN SHARON LEA ET AL) 12 January 1999 (1999-01-12) the whole document	1-68
Y	WO 00/44400 A (RTP PHARMA INC ; GEN HOSPITAL CORP (US)) 3 August 2000 (2000-08-03) page 6, paragraph 4 page 8, paragraph 2 - page 9, line 2 page 25; table 1 claims 1-18  ----- -/-	1-68

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search  29 September 2004	Date of mailing of the international search report  05/10/2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Fayos, C

# INTERNATIONAL SEARCH REPORT

national Application No  
T/CA2004/000648

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YAMAOKA T ET AL: "Development of pancreatic islets (review)." INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE. MAR 1999, vol. 3, no. 3, March 1999 (1999-03), pages 247-261, XP009037276 ISSN: 1107-3756 abstract page 254, column 1, paragraph 3 page 254, column 2, paragraph 4	1-68
Y	LOGSDON CRAIG D ET AL: "Adenoviral-mediated gene transfer of dominant negative ras inhibits pancreatic acinar cell growth responses to cholecystokinin and fibroblast growth factor" GASTROENTEROLOGY, vol. 112, no. 4 SUPPL., 1997, page A458, XP009037266 & DIGESTIVE DISEASE WEEK AND THE 97TH ANNUAL MEETING OF THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION; WASHINGTON, D.C., USA; MAY 11-14, 1997 ISSN: 0016-5085 sentence 1 - sentence 3	1-68



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA2004/000648

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 23-28, 31-36, 40-62 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
T/CA2004/000648

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5858977	A	12-01-1999	AU 681546 B2	28-08-1997
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			SK 43197 A3	10-03-1999
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			CN 1168678 A	24-12-1997
			CZ 9700981 A3	11-11-1998
			CZ 9701050 A3	14-10-1998
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			DE 69530403 T2	30-10-2003
			DK 785948 T3	04-08-2003
			EE 9700081 A	15-10-1997
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